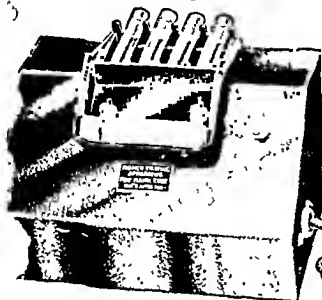




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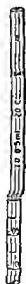
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*Fig. 9  
Pipette B*



*Fig. 10  
Pipette C*

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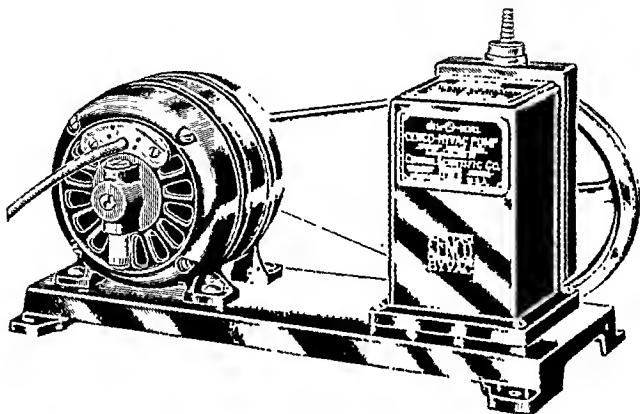
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# *The Journal of Laboratory and Clinical Medicine*

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VOL 21

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No 1

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## *CLINICAL AND EXPERIMENTAL*

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### THE INFLUENCE OF DEXTROSE INGESTION ON AMINO ACID NITROGEN, UREA NITROGEN AND HEMOGLOBIN CONCENTRATION OF THE BLOOD\*

---

E G SCHMIDT, PH D, AND J S EASTLAND, M D, BALTIMORE, MD

---

**D**URING the last few years we have had occasion to study the influence of infection upon the tolerance for dextrose<sup>1</sup> In addition a comparative study of dextrose and sucrose tolerance tests in normal and hospitalized subjects has been made<sup>2</sup> Since little information is available as to the effect of ingested dextrose upon the amino acid and urea content of the blood in health and in disease, the sugar tolerance studies were expanded in order to secure data on these points also In addition we found it both interesting and necessary to study the changes in blood volume, as measured by hemoglobin determinations, during the course of the tolerance tests

In an experiment upon a normal human subject Folin and Berglund<sup>3</sup> found that the ingestion of glucose depresses the postabsorptive level of amino acids, urea, and nonprotein nitrogen in the blood Katavama<sup>4</sup> reported that the ingestion of glucose by 5 subjects resulted in a fall in blood nonprotein nitrogen in two cases and an increased output of urine nitrogen whereas in two other cases the blood nonprotein nitrogen rose with a fall in the urinary nitrogen According to Milheiro<sup>5</sup> the amino acid nitrogen of the blood decreases after eating Bruger and Mirsky<sup>6</sup> performed 60 dextrose tolerance tests on 54 unselected subjects They state "(a) The urea nitrogen content of the blood may rise or (and) fall or remain unchanged A gradual fall in

---

\*From the Biochemical and Medical Departments of Mercy Hospital and the Departments of Biological Chemistry and Medicine of the University of Maryland School of Medicine

Received for publication March 13 1935

The authors wish to express their indebtedness to Sister M Joan d'Arc Sister Claude Rader Fleiner Baer and Francis Donovan for their very valuable assistance.

the urea nitrogen (1 to 7 mg. per cent) was observed in 22 out of 45 cases. A definite decrease (3 mg. per cent or more) occurred more often in subjects with diminished carbohydrate tolerance than in those with normal tolerance. (b) The total nonprotein nitrogen of the blood may rise or (and) fall or show no marked change. . . .'' Eveleth<sup>7</sup> found that the amino acid nitrogen content of the blood of pigs rose following glucose ingestion. In addition the urea usually increased although the average values showed practically no change.

The amino acids of the blood have been shown to be sensitive, also, to the injection of insulin. Thus Luek, Morrison, and Wilbur<sup>8</sup> proved that the injection of insulin lowers the amino acid nitrogen content of the blood of rabbits, rats, and human beings. Similar observations are reported by other workers.<sup>9-31</sup>

#### THE INFLUENCE OF INGESTED DEXTROSE ON THE AMINO ACID NITROGEN AND UREA NITROGEN CONTENT OF THE BLOOD

*Methods.*—About 6 or 7 c.c. of blood was secured by venipuncture and transferred to a small glass jar containing 10 mg. of dry, finely divided potassium oxalate. The protein-free filtrate was immediately prepared according to Haden.<sup>32</sup> The blood sugar was then determined according to Benedict's method,<sup>33</sup> the urea nitrogen, with some modifications,<sup>34</sup> by direct nesslerization.<sup>35, 36</sup> The amino acid nitrogen content of the blood filtrates was determined by the colorimetric method of Folin<sup>37</sup> using sodium beta-naphthoquinone 4-sulphonate.<sup>38</sup> During the time that the present work was in progress Folin's method has been subjected to considerable criticism both favorable and unfavorable<sup>39-47</sup> and has undergone important modifications.<sup>48-50</sup> Hence considerable uncertainty exists at present regarding the actual amount of amino acid nitrogen in the blood under normal, experimental and pathologic conditions. We were not interested primarily in the absolute amount of amino acid nitrogen in the blood but in the relative quantities which were present during the course of the dextrose tolerance tests. For this purpose Folin's original method proved very satisfactory.

The question whether fasting per se or frequent removal of blood influences the amino acid nitrogen level seems to be somewhat in dispute.<sup>10, 15, 18, 19, 51-55</sup> Therefore we decided to run some control experiments on fasting patients. After the customary overnight fast 6 to 8 c.c. of blood was collected in the usual manner at hourly intervals during the morning. The patient ate nothing during this period although in some cases 300 c.c. of water was given to drink at 8 A.M. Blood sugar, urea nitrogen, amino acid nitrogen, uric acid, and hemoglobin were determined on each sample. Thus these control determinations were made at the same time intervals as when the various blood constituents were determined during the tolerance tests. The average values which are given at the foot of Table I prove decisively that no appreciable variations in these constituents occur during the course of the morning following a twelve-hour fast, nor are they in-

fluenced by frequent blood withdrawals. Our findings do not substantiate the conclusions recently drawn by Pasehkir<sup>31</sup>

#### COMMENT

The general procedure followed in the administration of the dextrose has been described elsewhere.<sup>1</sup> Eighty seven individuals in all have been subjected to dextrose tolerance tests. In order to conserve space the data on each individual case have been omitted. The cases have been separated into 5 groups or classifications: 16 essentially normal individuals, miscellaneous diseases, 21 cases, arthritis and rheumatoid conditions, 12 cases, infection, 19 cases, diabetes mellitus, 19 cases. In this manner beginning with the normal curves in the first group the blood sugar values become more pathologic until the hyperglycemia is also accompanied by the glycosuria characteristic of diabetes mellitus in the last group. The average value for the blood sugar, amino acid nitrogen and urea nitrogen determinations at each period of the test have been summarized (Table II).

An examination of the above summarized data plainly indicates that an appreciable consistent decrease in amino acid nitrogen and urea nitrogen occurs during the course of a glucose tolerance test. The decrease in these nitrogenous blood constituents seems to be independent of the nature of the disease or of the degree of hyperglycemia. Thus the average maximum decrease in amino acid nitrogen for each group of diseases which occurred during the course of the tests is as follows: normal cases 12.91 per cent, miscellaneous diseases 9.55 per cent, arthritis and rheumatoid conditions 10.22 per cent, infection 14.68 per cent and diabetes mellitus 11.51 per cent. These values check fairly well with the average maximum decrease in amino acid nitrogen (12.91 per cent) which occurred in the blood of normal individuals following the ingestion of glucose. The average maximum decrease in this constituent for all 87 cases was 11.78 per cent. In addition it can readily be seen that the greater decrease in amino acid nitrogen occurred during the first hour of the test period: 53.0 per cent, 32.6 per cent of the decrease took place during the second hour and 14.4 per cent during the third hour of the tolerance test. There seems to be no correlation between the nature of the disease or the degree of hyperglycemia and the decrease in the amino acid nitrogen produced by the ingestion of dextrose.

As regards the urea nitrogen changes following dextrose ingestion quite similar results were secured. Thus the average maximum decrease in blood urea nitrogen which occurred in each group of cases following the administration of dextrose is as follows: normal cases 13.57 per cent, miscellaneous diseases 13.70 per cent, arthritis and rheumatoid conditions 14.78 per cent, infection 15.02 per cent and diabetes mellitus 11.00 per cent. Again there seems to be no significantly distinguishable difference between the percentage decrease in urea nitrogen for the group of normals and for the various groups of pathologic cases. The average maximum decrease in urea nitrogen for the 87 cases was 13.04 per cent, practically the same as that noted for the amino acid nitrogen (11.78 per cent). The greater decrease in the urea

TABLE I  
VARIATIONS IN THE BLOOD CONSTITUENTS DURING THE FASTING CONTROL PERIOD

WARD NUMBER	TIME A.M.	BLOOD SUGAR MG./100 C.C.	UREA NITROGEN MG. PER 100 C.C.			AMINO ACID NITROGEN MG./100 C.C.	URIC ACID MG./100 C.C.	HEMOGLOBIN PER CENT	DIAGNOSIS
			A	B	AVERAGE				
K 8	8	91	7.9	7.9	7.9	5.9	3.33	100.0	Streptococcal bronchitis
	9	94	8.0	7.7	7.9	5.8	3.33	93.0	
	10	90	7.6	7.0	7.3	5.9	3.44	96.6	
	11	94	7.7	7.8	7.8	5.8	3.48	94.4	
K 6	8	93	16.6	16.2	16.4	6.3	3.64	100.0	Chronic nephritis with hypertension
	9	92	17.2	15.8	16.5	6.4	3.56	102.0	
	10	90	16.8	16.1	16.5	6.4	3.51	100.0	
	11	94	16.0	15.3	15.7	6.1	3.69	98.5	
A 21	8	95	8.6	8.0	8.3	5.8	3.81	100.0	Acute bursitis
	9	96	8.3	8.0	8.2	5.9	3.64	100.0	
	10	98	8.2	7.6	7.9	5.9	3.64	101.5	
	11	99	8.3	8.3	8.3	6.0	3.86	98.0	
A 5	8	95	12.3	12.1	12.2	6.4		100.0	Gonococcal arthritis
	9								
	10	91	12.8	12.4	12.6	6.4		98.5	
	11		12.6		12.6	6.5		100.0	
A 10	8	102	8.6	8.4	8.5	6.5	4.70	100.0	Tinea saginita
	9	104	8.5	8.1	8.3	6.3	4.85		
	10	104	8.3	8.3	8.3	6.5	4.85	101.5	
	11	95	8.1	8.3	8.2	6.4	4.70	102.0	

TABLE I—CONT'D

WARD NUMBER	TIME A M	BLOOD SUGAR MG/100 CC	UREA NITROGEN MG PER 100 CC			AMINO ACID NITROGEN MG/100 CC	URIC ACID MG/100 CC	HEMOGLOBIN PER CENT	DIAGNOSIS
			A	B	AVERAGE				
O 10	8	99	102	106	104	64	346		Prostatitis
	9	99	99	104	102	62	370		
	10	102	99	103	101	63	365		
	11	105	102	102	102	62	352		
A 9	8	91	142	136	139	68	400	100 0	Cerebral hemorrhage (apoplexy)
	9	89	138	140	139	65	420	99 1	
	10	91	143	135	139	68	420	90 7	
	11	90	140	138	139	68	410	98 1	
A 1	8	91	231	242	237	54		100 0	Chronic arthritis
	9	87	234	234	234	50		104 0	
	10	90	231	231	231	54		102 0	
	11	89	231	230	231	55		99 6	
Average of above	8	94 6			12 66	61 9	383	100 0	
	9	91 6			12 63	61 0	388	99 7	
	10	94 5			12 46	62 0	388	99 6	
	11	94 9			12 53	61 7	389	99 7	

TABLE II

NUMBER OF CASES	CONDITION OF PATIENTS	BLOOD SUGAR MG./100 C.C.				AMINO ACID N. MG./100 C.C.				MAXIMUM DIFFERENCE PER CENT				UREA N. MG./100 C.C.				MAXIMUM DIFFERENCE PER CENT					
		F.	1 HR.	2 HR.	3 HR.	F.	1 HR.	2 HR.	3 HR.	F.	1 HR.	2 HR.	3 HR.	F.	1 HR.	2 HR.	3 HR.	F.	1 HR.	2 HR.	3 HR.		
16	Normal cases	99.4	140.9	113.7	95.4	6.35	6.00	5.69	5.83	12.91	11.57	10.74	10.40	10.00	13.57	12.75	12.45	12.06	13.37				
21	Misc. diseases	100.1	163.8	137.9	113.8	6.28	5.93	5.71	5.68	9.55	13.50	12.55	11.65	12.06	13.70	12.55	12.45	12.06	13.70				
12	Arthritis, etc.	102.1	197.1	177.2	136.8	6.26	5.79	5.62	5.67	10.22	10.42	10.19	9.13	8.88	14.78	10.42	10.19	9.13	8.88				
19	Infection	107.6	205.2	191.6	137.7	6.20	5.73	5.49	5.29	14.68	11.05	10.20	9.67	9.39	15.02	11.05	10.20	9.67	9.39				
19	Diabetes mellitus	117.5	232.3	225.8	174.8	6.34	6.04	5.80	5.61	11.51	10.59	10.18	9.53	9.35	11.00	10.59	10.18	9.53	9.35				
87	Average of above					6.29	5.90	5.66	5.55	11.78	11.43	10.77	10.08	9.94	13.04	11.43	10.77	10.08	9.94				
	Average hourly decrease (milligrams)						53.0	0.39	0.24	0.106							0.66	0.69	0.14				
	Average rate of decrease (per cent)																44.3	46.3	9.4				

nitrogen for all the cases occurred during the first and second hour of the test, namely 44.3 per cent and 46.3 per cent, respectively, and 9.4 per cent for the third hour period, whereas the greater decrease in amino acid nitrogen occurred during the first hour period. There seems to be no correlation between the disease or condition of the patient, the degree of hyperglycemia attained during the course of the test, and concomitant glycosuria if any, and the decrease in urea nitrogen.

In the later part of the present work data are given on the blood volume changes, as measured by relative hemoglobin determinations, which occurred during the course of these tests. Disregarding individual variations no consistent change in blood volume was noted despite the degree of hyperglycemia. Apparently the consistent decrease in amino acid nitrogen and urea nitrogen which we found to follow the alimentary ingestion of dextrose cannot be attributed to mere blood dilution.<sup>56</sup> However, it seems reasonable to believe that the decrease in these nitrogenous constituents of the blood observed during the course of the tolerance tests is due to the protein-sparing action<sup>57</sup> of the ingested dextrose. While the injection of insulin also results in a decrease in the amino acid nitrogen content of the blood, it is accompanied by an increase in blood urea. Since alimentary dextrose depresses both the amino acid and the urea content of the blood, it seems apparent that different metabolic factors are involved in each case. This is probably the explanation for the fact that simultaneous administration of glucose with insulin does not prevent the fall in blood and tissue amino acid nitrogen noted by Luek and Spaulding<sup>24</sup> and Ralli and Tiber.<sup>25</sup>

#### ALIMENTARY HYPERGLYCEMIA, HEMOGLOBIN CONCENTRATION AND THE BLOOD VOLUME

The ability of the blood to maintain its constant internal environment has long been recognized. Changes in the water content of the blood under various experimental conditions have been frequently studied.<sup>58, 59</sup> In order to eliminate the possibility that the definite decrease in amino acid nitrogen and urea nitrogen, which we found to take place during the course of a sugar tolerance test, might be due to dilution of the blood, the accompanying study of relative blood volume changes was made.

*Hemoglobin Determinations—Accuracy of the Method*—A mixed, pooled sample of blood containing 10 mg of potassium oxalate per 6 cc of blood was shaken with a rotary motion and the well-mixed blood drawn into a 0.5 cc Ostwald pipette. The blood was then discharged into a 500 cc Erlenmeyer flask containing exactly 250 cc of 0.1 N HCl. In this manner 10 samples of the same blood were set up for hemoglobin determinations. The flasks were shaken several times during the morning and then allowed to stand at room temperature for about twenty-four hours when colorimetric comparisons were made. The preparations were allowed to stand for this length of time in order to permit the development of a constant amount of color. The colorimeter was first carefully adjusted with the contents of flask No. 1 which was used as a standard. Each of the nine remaining flasks was



matched against this sample. Five readings were taken for each determination, none differing by more than 0.5 mm. from each other. The greatest variation from the specimen which was used as a standard and set at 20 mm. was 0.3 mm. and the maximum variation between the ten determinations was 0.5 mm. By this method, of course, only the relative concentration of hemoglobin can be estimated.

*Hemoglobin Concentration—Diurnal Variations.*—A number of experiments were also performed to see whether appreciable variations in hemoglobin concentration occur in subjects during the course of the day. About 3 or 5 c.c. of the blood was taken from the patient by venepuncture at 9 A.M., 12 Noon, and 4 P.M. Hemoglobin determinations were made in triplicate as previously outlined. After twenty-four hours the 9 A.M. preparation was used as a standard and the specimens collected at 12 Noon and 4 P.M., were matched against it. Thirty-four individual patients were studied in this manner.

The detailed findings have been omitted. However, the data indicate that appreciable variations in the concentration of hemoglobin occur in individual subjects during the course of the day. On the other hand, the relative average hemoglobin concentration of all the patients showed a slight decrease of doubtful significance as the day advanced. Assuming the relative average hemoglobin concentration to be 100 per cent at 9 A.M., it was 99.9 per cent at 12 noon and 98.57 per cent at 4 P.M. The average decrease, therefore, amounts to 0.1 per cent and 1.43 per cent for the noon and evening periods, respectively.

Six cases out of thirty-four 12 noon specimens differed by more than 0.5 mm., or 2.5 per cent in hemoglobin, from their corresponding morning specimens. The maximum decrease in hemoglobin for any one subject was 8.76 per cent and the maximum increase noted in any case was 5.3 per cent during this period. Among the 4 P.M. samples, ten of the thirty-four determinations differed by more than 0.5 mm. or 2.5 per cent in hemoglobin, from their corresponding morning specimens. The greatest relative decrease in hemoglobin concentration during this period was 10.07 per cent and the maximum increase in any one case was 5.8 per cent. The greatest variation which occurred in any one patient during the day was 10.07 per cent. These results are essentially in agreement with those of Dreyer, Bazette and Pierce,<sup>81</sup> Rabinowitch,<sup>82</sup> and Chanutin, Smith, and Mendel.<sup>83</sup> From these observations we can conclude that, although variations as high as 10.07 per cent in hemoglobin concentration occur during the course of the day, the average diurnal variation in hemoglobin concentration, or blood volume, was insufficient to account for the consistent decrease in amino acid nitrogen and urea nitrogen observed during the course of the sugar tolerance tests.

*Hemoglobin Concentration—Variations During the Course of Dextrose Tolerance Tests.*—In a similar manner hemoglobin determinations were made on the blood specimens used for the preparation of the protein-free filtrates. These experiments were performed in order to determine whether the osmotic

effects of the glucose in the stomach and later in the blood would exert an appreciable influence upon the blood volume as measured by variations in hemoglobin concentration.<sup>26 28 31 30</sup>

Our experimental data were divided into two groups, one containing the findings on nineteen dextrose tolerance tests which yielded normal blood sugar curves and the other the findings on thirty five tests which yielded high, diabetic, or diabetic like blood sugar curves due to infection, diabetes mellitus or arthritis or allied rheumatoid conditions. The conclusions only will be reported. No consistent variation in the blood volume, as measured by relative hemoglobin percentages occurred regardless of the degree of glycemia developing during the course of the tolerance tests. Thus the average blood sugar values for the normal curves at the fasting, one, two, and three hour periods of the tests were 98.0, 141.4, 109.2, and 98.8 mg per 100 cc of blood, respectively. The corresponding average hemoglobin readings were 20.0 (set as standard), 19.97, 20.21 and 20.15 mm on 100.0, 100.15, 98.96, and 99.26 per cent hemoglobin. The average blood sugar values for the pathologic curves at each period of the test were 105.8, 206.5, 192.2, and 146.7 mg per 100 cc of blood. The corresponding average hemoglobin readings were 20.0 (set as standard), 19.95, 20.11, and 20.20 mm on 100.0, 100.26, 99.45, and 99.01 per cent hemoglobin. Obviously therefore no regular significant variation in blood volume occurred during the course of the dextrose tolerance tests regardless of the degree of hyperglycemia. Hence the decrease in nitrogenous constituents of the blood (amino acids and urea) following the ingestion of dextrose is not due to blood volume changes.

#### SUMMARY

1. Eighty seven individual cases have been subjected to dextrose tolerance tests during which the concentration of dextrose, amino acid nitrogen, urea nitrogen and hemoglobin of the blood have been determined. The data indicate that an appreciable decrease in the amino acid nitrogen and urea nitrogen content of the blood occurs during the course of dextrose tolerance tests. The cases have been separated into five groups. The average maximum decrease in amino acid nitrogen for each group was: normal cases 12.91 per cent, miscellaneous diseases 9.55 per cent, arthritis and rheumatoid conditions 10.22 per cent, infection 14.68 per cent, and diabetes mellitus 11.51 per cent. The average maximum decrease in urea nitrogen for these same groups was: normal cases 13.57 per cent, miscellaneous diseases 13.70 per cent, arthritis and rheumatoid conditions 14.78 per cent, infection 15.02 per cent and diabetes mellitus 11.00 per cent. The average maximum decrease in urea nitrogen for the 87 cases was 13.04 per cent practically the same as the average maximum decrease in amino acid nitrogen (11.78 per cent). No significant variations were noted between the results for the normal group and the pathologic groups. No correlation was observed between the various diseases or the degree of hyperglycemia attained during the course of the tests and the decrease in amino acid nitrogen and urea nitrogen content of the blood.

2. The concentration of blood dextrose, amino acid nitrogen, urea nitrogen, uric acid, and hemoglobin of control patients remained unchanged during the morning following a twelve-hour fast, despite hourly withdrawals of blood.

3. Diurnal variations in hemoglobin concentration up to 10 or 11 per cent were noted. The average values remained constant however. No relation between the degree of hyperglycemia and blood volume changes as determined by the relative hemoglobin concentration was noted during course of the glucose tolerance tests. Except for individual variations, the blood volume or hemoglobin concentration remained unchanged during the tests.

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## A COMPARATIVE STUDY OF GLUCOSE AND SUCROSE TOLERANCE TESTS\*

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THE glucose tolerance test or perhaps more correctly the blood sugar time curve<sup>1</sup> (1933), has acquired justified popularity since it is the most sensitive test available for the detection and evaluation of disorders of carbohydrate metabolism. While the metabolism of glucose both under normal and pathologic conditions has been studied extensively little attention has been given to sucrose metabolism, especially of the hospitalized patient.

Although glucose is utilized in every modern hospital particularly for patients requiring intravenous therapy it is not so readily available to the practicing physician particularly those in the smaller communities. Cane sugar, sucrose however may be readily obtained pure and is much less expensive. In view of these facts a comparative study of the blood sugar curves, and sugar excretion if any was made on a series of 57 hospital patients after the ingestion of glucose followed a few days later by an equal quantity of sucrose or vice versa.

### HISTORICAL RESUME

The literature on sucrose metabolism is not voluminous. According to Rohman and Nagano (1907) sucrose is rapidly hydrolyzed in the alimentary canal into its two component constituents glucose and fructose which upon absorption produce a rise in the blood sugar. Similar observations have been made by von Noorden<sup>2</sup> (1917) Jacobsen<sup>3</sup> (1913) Eisner and Foister (1921) and Frank and Mehlhorn<sup>4</sup> (1920). Whereas Worm Müller<sup>5</sup> (1884) noted sucrose in the urine after the administration of 50 to 250 gm of this sugar other workers<sup>6</sup> usually report glucose as the sugar being present. Schatt<sup>7</sup> (1923) found that the blood sugar of normal individuals who had ingested 20 gm of cane sugar rose as rapidly as with glucose or levulose although to a lesser height. Field<sup>8</sup> (1919) fed 100 gm of sucrose to normal colored males. The average rise in blood sugar was 20 mg per 100 cc. Moutier and Camus<sup>9</sup> (1929) have compared glucose and sucrose tolerance tests in one diabetic and in six nondiabetic individuals. Pusek<sup>11</sup> (1931) has studied the influence on diabetic patients of injections of sucrose and invert sugar upon ketonuria ketonemia and intravenously injected insulin. Greenwald and Pennell<sup>12</sup> (1930) noted that in a series of 15 infants (two to ten days old) when fed 2 gm of dextrose per kilo body weight all but one gave a prompt

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TABLE I  
COMPARISON OF GLUCOSE AND SUCROSE TOLERANCE TESTS IN DIABETES MELLITUS

CASE	HOSPITAL NUMBER	AGE	SEX	GLUCOSE TOLERANCE TESTS				SUCROSE TOLERANCE TESTS				DIAGNOSIS
				DATE	TIME HR.	BLOOD SUGAR MG. PER 100 C.C.	URINE SUGAR	DATE	TIME HR.	BLOOD SUGAR MG. PER 100 C.C.	URINE SUGAR	
19	Out	43	M	3/23/33	F. 1 2 3	167 435 480 367	+ +++ +++ +++ (14.3 gm.)	4/ 1/33	F. 1 2 3	179 333 333 250 (14.7 gm.)	+ ++ +++ +++ (14.7 gm.)	Diabetes mellitus
20	3858	46	M	1/ 6/33	F. 1 2 3	114 308 286 233	Neg. Tr. +++ +++	1/19/33	F. 1 2 3	110 250 182 154	Neg. +++ +++ +++	Diabetes mellitus, emphysema, chronic bronchitis, arthritis spine (early)
21	9114	54	M	4/24/33	F. 1 2 3	137 250 282 277	Neg. Neg. ++ +++ (1.56 gm.)	4/28/33	F. 1 2 3	130 250 263 222 (0.64 gm.)	Neg. Neg. + ++	Diabetes mellitus, arteriosclerotic gangrene foot, caries, chronic bronchitis
22	3856	42	F	10/29/31	F. 1 2 3	100 286 190 95	Tr. +++ +++ +++	11/ 4/31	F. 1 2 3	118 235 159 99	Neg. +++ ++ Neg.	Diabetes mellitus, arteriosclerotic cardiovascular disease
23	7874	70	M	10/29/32	F. 1 2 3	125 211 200 198	Neg. Neg. + +	11/ 5/32	F. 1 2 3	118 238 222 187	Neg. Neg. + +	Diabetes mellitus, bilateral cataracts, fracture upper right tibia
24	14073	55	F	7/13/34	F. 1 2 3	109 235 270 286	Neg. Neg. + Tr.	7/24/34	F. 1 2 3	123 233 256 244	Neg. Neg. + Tr.	Diabetes mellitus, obesity, coronary thrombosis, arteriosclerosis

TABLE 1—CONT'D

CASE	HOSPITAL NUMBER	AGE	SEX	GLUCOSE TOLERANCE TESTS				SUCROSE TOLERANCE TESTS				DIAGNOSIS
				DATE	TIME HR	BLOOD SUGAR MG PER 100 CC	URINE SUGAR	DATE	TIME HR	BLOOD SUGAR MG PER 100 CC	URINE SUGAR	
25	13045	30	F	7/26/34	F 1 2 3	114 222 169 114	Neg Neg + Neg	7/27/34	F 1 2 3	106 225 138 110	Neg + Neg Neg	Diabetes mellitus, menopause
26	14104	35	M	7/14/34	F 1 2 3	111 245 133 110	Neg ++ +++ Neg	5/7/34	F 1 2 3	100 167 103 103	Neg + + Neg	Diabetes mellitus, cerebral angio spasm, pterygium right eye
27	9799	45	M	4/8/33	F 1 2 3	119 175 191 145	Neg Neg + +	4/11/33	F 1 2 3	118 202 205 114	Neg + ++ ++++	Diabetes mellitus, infected teeth dental caries
28	Out	25	M	11/5/32	F 1 2 3	105 178 121 119	Neg +++ Tr Neg	3/4/33	F 1 2 3	105 154 118 111	Neg ++ + Tr	Potential diabetes
29	9342	46	M	2/25/33	F 1 2 3	95 227 241 165	Neg Neg + +	2/28/33	F 1 2 3	98 206 142 100	Neg Neg + Neg	Diabetes mellitus detachment of teeth and eleventh costal carti lage
30	Out	40	M	6/4/34	F 1 2 3	118 211 117 111	Neg + ++ Neg	6/7/34	F 1 2 3	111 211 87 81	Neg Neg ++ Tr	Diabetes mellitus (0.40 gm.)



eign body, unchanged. While the above experiment was performed with ordinary fasting blood it does not seem probable, in view of the work of Abderhalden and Buadze (1932), that the ingestion of cane sugar could effect

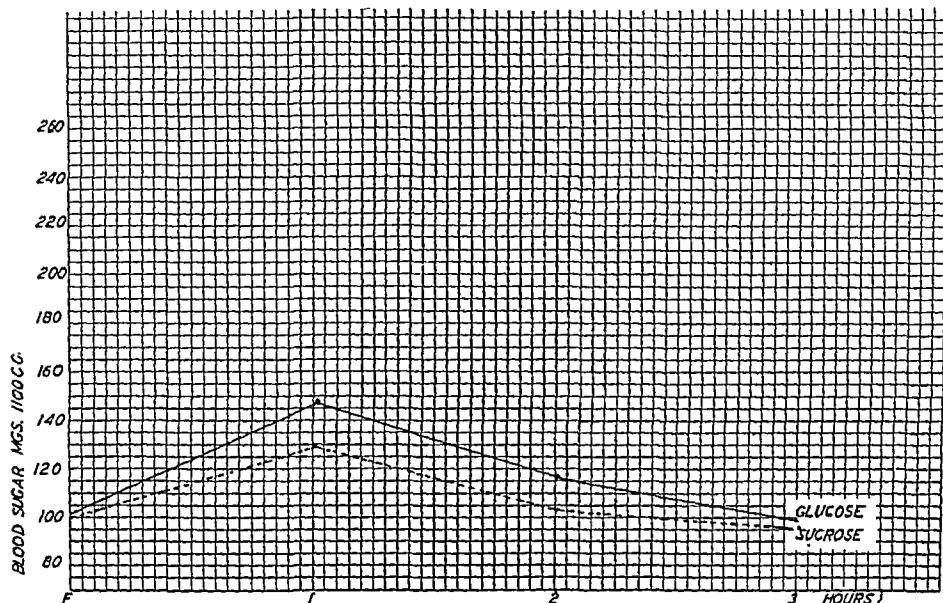


Chart 1.—Normal blood sugar curves following the ingestion of glucose or sucrose (1.5 gm. per kilo). Average of 18 separate cases.

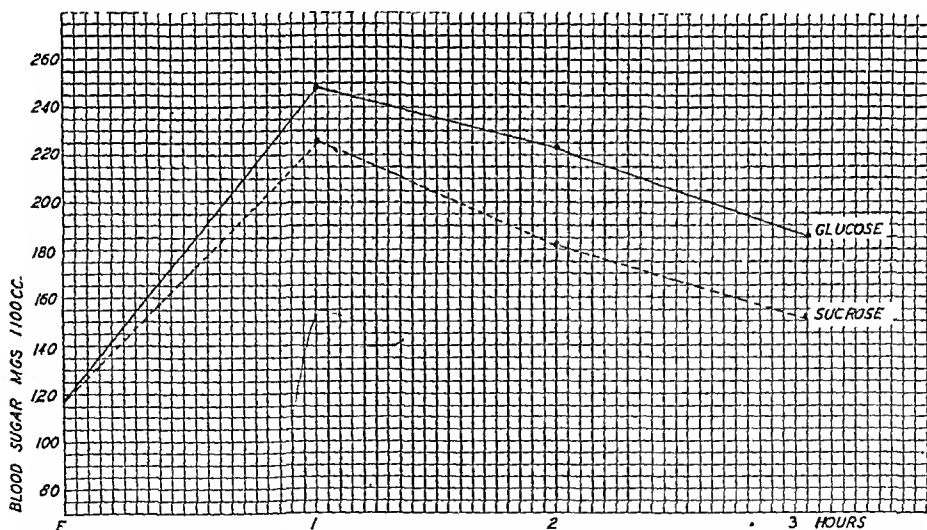


Chart 2.—Blood sugar curves in diabetes mellitus following the ingestion of glucose or sucrose (1.5 gm. per kilo). Average of 12 separate cases.

the mobilization of appreciable quantities of sucrase into the blood stream within a few minutes.

#### RESULTS

During the course of this investigation glucose and sucrose tolerance tests have been carried out upon 57 separate hospital patients. No attempt

has been made to secure a series of normal controls, since we feel that other workers have amply demonstrated the fact that healthy individuals, upon the ingestion of cane sugar, yield blood sugar time curves well within the normal range.

The data have been collected into three groups. The first group (Chart 1) contains the summarized data on eighteen cases which, although pathologic in nature, yielded relatively normal blood sugar curves with both glucose and sucrose. Table 1 and Chart 2 contain the experimental findings on twelve cases of diabetes mellitus. With one exception (No. 19) these patients were admitted for conditions other than diabetes and only through tolerance studies were the diabetic tendencies discovered. Due to the disturbing influence of large quantities of sugar we have hesitated to subject known diabetic patients to these tests. The third group of patients (Table II and Chart 3) includes twenty-seven cases involving infections or arthritic conditions of

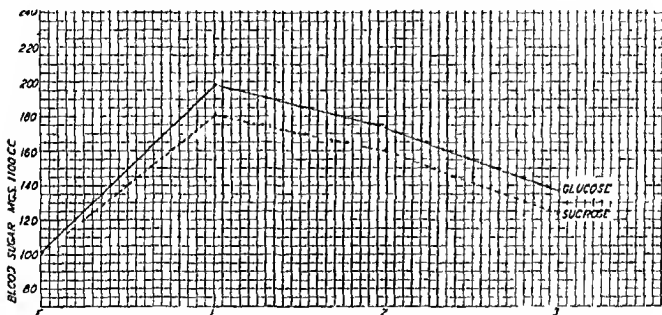


Chart 3—Blood sugar curves in infectious arthritis etc. following the ingestion of glucose or sucrose (1, 2, 4, 6, 10, 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220, 240 mg/100 cc). Average of 27 separate cases.

various types which, in general, yielded abnormally high blood sugar time curves with both sugars.

In studying the data given by these two tolerance tests we were interested primarily in discovering whether both sugars give the same type of blood and urine sugar curves. In short, will the ingestion of cane sugar yield the same clinical information as a like quantity of glucose?

#### COMMENT AND SUMMARY

1. Fifty-seven hospital cases have been subjected to both glucose and sucrose tolerance tests and the respective blood and urine sugar curves studied comparatively. While individual differences were found between the various blood sugar curves, their general pattern was similar. A group of eighteen patients (Chart 1), free from obvious carbohydrate metabolism abnormalities, gave normal blood sugar curves and urines negative to Benedict's solution with both sugars. The average blood sugar values following sucrose ingestion were 100.7, 129.2, 102.3, and 95.5 mg per cent for the fast-

TABLE II  
COMPARISON OF GLUCOSE AND SUCROSE TOLERANCE TESTS IN INFECTIONS AND ALLIED CONDITIONS

CASE	HOSPITAL NUMBER	AGE	SEX	GLUCOSE TOLERANCE TESTS					SUCROSE TOLERANCE TESTS					DIAGNOSIS
				DATE	TEMP. ° F.	TIME HR.	BLOOD SUGAR MG. PER 100 C.C.	URINE SUGAR	DATE	TEMP. ° F.	TIME HR.	BLOOD SUGAR MG. PER 100 C.C.	URINE SUGAR STC. R	
31	8438	55	M	12/ 3/32	98.6	F. 1 2 3	127 286 253 197	Neg. Neg. Neg. Neg.	11/30/32	98.6	F. 1 2 3	100 200 204 147	Neg. Neg. Neg. Neg.	Hypertensive cardiovascular disease, tertiary syphilis, bronchitis, left hydrocele
32	8311	54	F	11/21/31	100.0	F. 1 2 3	125 250 250 199	Neg. Neg. Neg. Tr.	11/21/32	100.0	F. 1 2 3	114 222 201 116	Neg. Neg. Neg. Neg.	Infectious arthritis, earies pyorrhea, chronic tonsillitis
33	7983	25	M	10/ 3/32	98.6	F. 1 2 3	100 211 175 111	Neg. Neg. Neg. Neg.	10/ 6/32	98.6	F. 1 2 3	105 230 161 95	Neg. Neg. Neg. Neg.	Syphilis, secondary anemia, agranu- loeytic anemia (convalescent period)
34	6407	71	M	6/ 9/32	100.2	F. 1 2 3	98 142 217 204	Neg. Neg. Neg. Neg.	6/13/32	99.2	F. 1 2 3	100 167 208 184	Neg. Neg. Neg. Neg.	Tenosynovitis right shoulder, puru- lent otitis media
35	6362	70	M	6/ 7/32	98.2	F. 1 2 3	91 241 217 190	Neg. Neg. ++++ ++++	6/13/32	98.6	F. 1 2 3	91 200 192 167	Neg. Neg. Neg. Neg.	Multiple infectious arthritis, chronic infected tonsils
36	5796	40	F	4/23/32		F. 1 2 3	95 190 152 138	Neg. Neg. Neg. Neg.	4/29/32		F. 1 2 3	93 134 125 88	Neg. Neg. Neg. Neg.	Infectious arthritis, tonsillitis, pyor- rhea, alveolaris
37	8396	53	M	12/21/32	98.6	F. 1 2 3	91 153 125 116	Neg. Neg. Neg. Neg.	11/30/32	98.6	F. 1 2 3	100 157 145 133	Neg. Neg. Neg. Neg.	Malux valgus deformity left foot, lymphangitis right knee

TABLE II—CONT'D

CASE	HOSPITAL NUMBER	AGE	SEX	GLUCOSE TOLERANCE TESTS					SUCROSE TOLERANCE TESTS					DIAGNOSIS
				DATE	TEMP °F	TIME HR	BLOOD SUGAR MG PER 100 CC	URINE SUGAR	DATE	TEMP °F	TIME HR	BLOOD SUGAR MG PER 100 CC	URINE SUGAR	
38	4270	52	M	1/11/32	98.4	1	91	Neg	1/7/32	99.6	1	91	Neg	Acute pulmonary tuberculosis, pleurisy
						1	187	Neg			1	222	Neg	
						2	174	Neg			2	142	Neg	
						3	87	Neg			3	80	Neg	
39	13032	55	F	4/12/34	98.8	F	94	Neg	4/14/34	99.0	F	108	Neg	Hypertrophic arthritis right knee, syphilis, hypertensive cardiovascular disease
						1	238	Neg			1	231	Neg	
						2	227	Neg			2	167	Neg	
						3	179	Neg			3	145	Neg	
40	5071	42	M	2/4/33	99.8	F	100	Neg	2/2/33	99.4	F	102	Neg	Rheumatic cardiovascular disease with aortic and mitral insufficiency, subacute bacterial endocarditis, syphilis, lumpygra
						1	170	Neg			1	190	Neg	
						2	171	Neg			2	138	Neg	
						3	125	Neg			3	98	Neg	
41	9486	48	M	4/24/33	100.0	F	100	Neg	4/29/33	101.4	F	100	Neg	Pulmonary abscess, dental caries, pyorrhea
						1	125	Neg			1	142	Neg	
						2	123	Neg			2	118	Neg	
						3	110	Neg			3	114	Neg	
42	9433	58	F	3/7/33	98.9	1	106	Neg	3/11/33	98.9	F	106	Neg	Active pulmonary tuberculosis paroxysmal hypertension, mitral stenosis
						1	260	Tr			1	167	Tr	
						2	159	++			2	200	+++	
						3	122	++			3	128	++	
43	13166	41	M	2/9/34	98.6	F	99	Neg	2/12/34	99.2	F	97	Neg	Toxic polyneuritis, hyperkeratosis of feet
						1	206	Neg			1	163	Neg	
						2	133	Neg			2	192	Neg	
						3	109	Neg			3	142	Neg	
44	4312	35	F	1/29/32	98.6	F	111	Neg	1/26/32	98.6	F	105	Neg	Syphilis, typhoid fever (late convalescent period)
						1	230	Neg			1	190	Neg	
						2	160	Neg			2	133	Neg	
						3	90	Neg			3	91	Neg	

TABLE II—Cont'd

CASE	HOSPITAL NUMBER	AGE	SEX	GLUCOSE TOLERANCE TESTS					SUCROSE TOLERANCE TESTS					DIAGNOSIS
				DATE	TEMP. °F.	TIME HR.	BLOOD SUGAR MG. PER 100 C.C.	URINE SUGAR	DATE	TEMP. °F.	TIME HR.	BLOOD SUGAR MG. PER 100 C.C.	URINE SUGAR	
45	9435	67	M	3/ 6/33	98.6	F. 1 2 3	111 161 125 114	Neg. Neg. Neg. Neg.	3/ 9/33	98.6	F. 1 2 3	109 211 200 154	Neg. Neg. Neg. Neg.	Gout, generalized arteriosclerosis, coronary sclerosis
46	9312	29	M	3/ 9/33	99.8	F. 1 2 3	100 200 164 111	Neg. Neg. Neg. Neg.	2/28/33	99.8	F. 1 2 3	105 167 137 110	Neg. Neg. Neg. Neg.	Syphilitic cardiovascular disease, aneurism arch aorta, occlusion left main bronchus
47	7781	62	M	9/24/32	100.0	F. 1 2 3	128 200 213 198	Neg. Neg. Tr. Neg.	9/23/32	100.8	F. 1 2 3	160 308 333 303	Neg. Neg. Tr. Neg.	Cellulitis left leg
48	7781	62	M	10/21/32	98.6	F. 1 2 3	114 194 160 108	Neg. Neg. Neg. Neg.	10/18/32	98.6	F. 1 2 3	115 179 114 95	Neg. Neg. Neg. Neg.	Cellulitis left leg (recovery prae- tically complete)
49	3693	25	M	4/ 5/34	100.0	F. 1 2 3	108 154 127 121	Neg. Neg. Neg. Neg.	4/ 9/34	98.6	F. 1 2 3	88 95 96 91	Neg. Tr. Tr. Tr.	Recurrent lymphangitis leg, infected tonsils, acute infectious arthritis, (some improvement when suerosc test was performed)
50	13063	46	M	2/ 7/34	99.6	F. 1 2 3	83 154 150 133	Neg. Neg. Neg. Neg.	2/ 9/34	100.2	F. 1 2 3	108 135 156 105	Neg. Neg. Neg. Neg.	Cerebral vascular syphilis with hemi- plegia.
51	7741	40	M	2/ 5/33	99.0	F. 1 2 3	100 170 155 125	Neg. Neg. Neg. Neg.	2/ 2/33	99.0	F. 1 2 3	102 190 138 98	Neg. Neg. Neg. Neg.	Chronic arthritis

TABLE II—Cont'd

CASE	HOSPITAL NUMBER	AGE	SEX	GLUCOSE TOLERANCE TESTS				SUCROSE TOLERANCE TESTS				DIAGNOSIS		
				DATE	TEMP °F	TIME HR	BLOOD SUGAR MG PER 100 CC	URINE SUGAR	DATE	TEMP °F	TIME HR		BLOOD SUGAR MG PER 100 CC	URINE SUGAR
32	14213	41	M	5/12/34	100.0	1 2 3	95 185 136 116	Neg Neg Neg Neg	5/15/34	99.2	1 2 3	100 133 130	Neg Neg Neg	Chronic multiple arthritis, pleurisy with effusion, infected tonsils
33	13722	42	F	4/29/34	99.0	1 2 3	114 179 204 133	Neg Neg Neg + Neg	5/5/34	98.7	1 2 3	100 175 142 136	Neg Neg Neg Neg	Chronic multiple arthritis, colloid and adenomatous goiter, dental abscess
34	9446	34	M	3/6/33	98.6	1 2 3	100 136 118 91	Neg Neg Neg Neg	3/14/33	98.6	1 2 3	100 160 135 87	Neg Neg Neg Neg	Acute rheumatic fever chronic infected tonsils, chronic sinusitis
35	14029	51	M	4/23/34	100.0	1 2 3	108 250 222 174	Neg Neg Neg + + Tr	4/27/34	100.2	1 2 3	105 204 129 118	Neg Neg Neg + Neg	Acute infectious arthritis, chronic prostatitis, obesity, dental caries
36	14031	35	F	4/26/34	99.0	1 2 3	91 190 200 167	Neg Neg Neg + Neg	5/14/34	99.0	1 2 3	98 160 136 105	Neg Neg Neg Neg	Hypertensive cardiovascular disease, old hemiplegia
37	14030	42	F	7/21/34	99.0	1 2 3	119 164 200 148	Neg Neg Neg Neg	7/19/34	99.0	1 2 3	104 189 167 80	Neg Neg Neg Neg	Abdominal adhesions, distasteful recti

ing, one-hour, two-hour, and three-hour periods, respectively. These same patients gave, upon the administration of a similar amount of glucose (1.5 gm. per kilogram of body weight), blood sugar values of 101.4, 146.0, 117.2, and 98.2 per cent at the same time intervals. Thus the average blood sugar curve given by the sucrose meal was 11.51, 12.71, and 2.75 per cent lower than that given by glucose for the one-hour, two-hour, and three-hour periods, respectively. It is evident, therefore, that the ingestion of approximately 100 gm. of sucrose ordinarily yields a blood sugar curve well within the normal limits as established by glucose tolerance tests. The urines were free from sucrose or reducing sugars regardless of the type of sugar ingested.

2. Twelve patients with diabetes mellitus showed marked hyperglycemia and glycosuria with both sugars (Table I and Chart 2). The highest blood sugar value (Case 19) given by sucrose was 333 mg. per cent as compared to 480 mg. per cent given by the same patient when glucose was administered. The average blood sugar values for these sucrose tolerance tests were 118.0, 225.3, 184.0, and 149.6 mg. per cent for the fasting, one-hour, two-hour, and three-hour periods, respectively, as compared to 117.8, 247.8, 222.6, and 185.0 mg. per cent for glucose at the respective time intervals. Thus the blood sugar response to sucrose ingestion was 9.8, 17.34, and 19.13 per cent lower than that given by glucose for the one-, two- and three-hour intervals, respectively. Both sugars resulted in glycosuria to approximately the same extent. In no case did the administration of sucrose fail to bring out the diabetic tendencies of these patients. Hence it is our opinion that clinically sucrose is as satisfactory as glucose for the detection and evaluation of diabetes mellitus.

3 Twenty-seven patients with various arthritic and infectious conditions have also been studied in a like manner (Table II and Chart 3). The average blood sugar values following sucrose ingestion were 103.9, 183.4, 160.4, and 123.7 mg. per cent for the fasting, one-hour, two-hour, and three-hour periods, respectively, as compared to 103.7, 198.3, 173.8, and 136.9 mg. per cent for glucose at similar time intervals. Thus these patients showed a blood sugar response to sucrose ingestion 8.02, 7.71, and 9.64 per cent lower than the response given by glucose at the various periods of the test. However, one can readily conclude from the data that sucrose tolerance tests show as well as glucose tolerance tests that an abnormality in carbohydrate metabolism is present during arthritis, infections, and similar conditions. In addition, sucrose ingestion shows equally well that this abnormality disappears as the infection clears up. Material differences between consecutive blood sugar curves following ingestion of sugar need not always be surprising since recovery from the infection rapidly increases the patient's ability to remove sugar from the blood. It seems obvious that in some cases where material differences between the curves were noted, an important, clinically wise, in the condition of the patient could have taken place, although the data evident clinically. Case 47-48 is interesting in this respect.

on this patient clearly proves that sucrose tolerance tests bring out the improvement in carbohydrate tolerance as well as does glucose when the infection has cleared up

As a rule, despite the high values for the blood sugars, the urines were usually free from reducing sugars. Glycosuria was present during six glucose tolerance tests and during two sucrose tolerance tests. Case 35 differed materially in that glucose ingestion resulted in 4 plus urine sugar at the second and third hourly periods whereas the urine was free from sugar following the sucrose meal. The blood sugar curves were abnormally high with both sugars however. Whether glucose intake results in more frequent glycosuria than does sucrose can only be proved by more extensive studies.

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## THE BACTERICIDAL AND PHOTOCHEMICAL PROPERTIES OF IRRADIATED PETROLATUM AND MINERAL OIL\*

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IN 1931 Eising<sup>1</sup> suggested the use of irradiated petrolatum for the treatment of infected wounds and sinuses. He had observed that with this treatment the bacterial count in the discharge from the wounds diminished, the growth of granulation tissue was stimulated and healing was hastened. These effects were attributed to a bactericidal action together with a stimulus to healing, both of which he believed were due to secondary rays emanating from petrolatum following ultraviolet irradiation. He found that photographic plates could be sensitized to subsequent development if they were placed in the dark over petrolatum previously exposed to ultraviolet light from which the longer wave lengths had been filtered. Exposure occurred even though the plates were sealed between sheets of x-ray film from which the emulsion had been removed. From these observations he concluded that the therapeutic value of the oil was due to secondary radiant energy dependent on exposure to ultraviolet light rays. In subsequent articles<sup>2, 3</sup> he attributed the photochemical action of the irradiated petrolatum to heavy gaseous reducing agents and not to actinism. Even though infection apparently subsided in wounds which were treated, the petrolatum failed to kill bacteria in vitro. He remarks, however, that if a loop of *Staphylococcus aureus* from an agar slant is suspended finely in irradiated oil, according to the method of Thompson and Sheard,<sup>4</sup> subcultures from this oil show no growth after eight hours. He concludes that the bactericidal action differs from that of ordinary germicides in that it is slow, that the vapor affecting the photographic plate is not the same as the bactericidal vapor arising from some irradiated vegetable and animal oils, and that the factor which promotes healing may be distinct from that detrimental to the growth of bacteria.

While it has been impractical to carry out extensive laboratory experiments on the growth-stimulating qualities of irradiated petrolatum, the photochemical and bactericidal properties have been investigated. The effects on both aerobic and anaerobic bacteria have been studied. Unfortunately, so little if any bactericidal effect has been observed, that the correlation between the bactericidal and photosensitizing qualities could not be investigated.

### PHOTOCHEMICAL EFFECTS

The first few experiments confirmed some but not all of the observations previously recorded in the literature. Normal petrolatum failed to fog photographic plates, but irradiated petrolatum did fog them within twenty-four hours.

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Glass or quartz placed between the surface of the petrolatum and the emulsion prevented fogging, but the interposition of a film of acetocellulose failed to abolish the effect. In these and all subsequent exposures, the petrolatum was irradiated under a quartz mercury arc at 30 cm. in flat dishes with uniform surface exposures. The photochemical effects were tested with 10 cc. of irradiated oil in Stender jars of uniform size. Eastman hypersensitive press plates were inverted over these jars for twenty-four hours.

After confirmation of the observation that plates were affected through washed wax film but not through glass or quartz, the assumption that the fogging effect resulted from vapor from the irradiated petrolatum was subjected to experimental study. A Stender jar was partially filled with irradiated petrolatum, a plate was sealed to the rim with gelatin, and a current of air was drawn rapidly over the surface of the petrolatum through an inlet and outlet sealed in opposite sides of the jar. The plate was unfogged after twenty-four hours. Both normal and irradiated petrolatum were then alternately degassed in a vacuum and saturated with carbon dioxide, nitrogen or oxygen. If petrolatum previously activated by ultraviolet light was washed successively with any one of these gases, samples removed after each washing showed a steady decrease in the photochemical effect. Six washings usually abolished the effect entirely. Normal oil similarly degassed and washed repeatedly with nitrogen or carbon dioxide was not activated by ultraviolet light after several washings, even if finally saturated with oxygen before irradiation. If oxygen was used for washing, samples removed after each degassing and irradiated showed a less rapid decrease in photochemical effect, when compared with similar samples washed with carbon dioxide and saturated once with oxygen. More washings were required with oxygen than with carbon dioxide to abolish completely the fogging effect. A current of air bubbled through irradiated mineral oil partially removed the substance responsible for fogging. On the assumption that this substance was vaporized air was bubbled through several hundred cubic centimeters of irradiated mineral oil, collected as it escaped from the oil, conducted through a 1 mm. quartz tube lying against the emulsion of a photographic plate and then allowed to escape from a capillary tube against the surface of a film of acetocellulose pressed firmly against a second plate. The first plate was unexposed where the tube lay against it, the second was fogged where the air current flowed against the film of acetocellulose. These experiments seem to show conclusively that the fogging effects obtained with irradiated petrolatum are caused by a vapor which is readily diffusible and must come in contact with the emulsion to affect the plate.

In one of the previous experiments, oil fully saturated with oxygen previous to irradiation appeared more photoactive than a sample less fully saturated. This experiment suggested that oxygen might be necessary for the activation of the inactive substance. An ideal experiment was impossible. Manipulation designed to completely remove the oxygen prior to irradiation removed the precursor of the substance responsible for fogging as well. As a compromise the effects of practically equivalent saturation with oxygen and nitrogen prior to radiation were compared. A sample of normal oil was degassed and satu-

rated twice with carbon dioxide. This reduced the fogging effect on plates to a point where comparisons could be readily made. Equal volumes of the oil, still saturated with carbon dioxide, were transferred to two identical vessels. The vessels were covered with clear cellophane. Equal volumes of nitrogen and oxygen were then bubbled through these samples at equal rates while the samples were exposed similarly to ultraviolet light. The sample treated with nitrogen was photochemically inactive but intense activity was obtained with the oxygen-treated sample, proving the necessity for oxygen in the process of activation.

With the well-recognized oxidizing effect of ultraviolet light in mind, the dependence on oxygen for the photochemical activation of the oil suggested that active organic peroxides might be formed. Harris, Bunker, and Milas<sup>5, 6</sup> have found peroxides in the vapor above irradiated oils. Tests for peroxidic substances immediately above the surface of irradiated petrolatum confirmed their observations. They found no aldehydes, nor did they obtain diffusion of peroxides through x-ray film. Technical difficulties have rendered tests for aldehydes in the vapor immediately above active oils inconclusive. Experiments have shown, however, that the peroxides diffuse through a film of acetocellulose and that aldehydes are to be found with them. Sections of washed x-ray film were coated with pure gelatin. When the coatings were nearly dry the covers of Petri dishes were pressed rim down on the films of gelatin. The covers with the adhering sections of x-ray film were then placed over the bottoms of the dishes which were partially filled with petrolatum. Some of the gelatin films were plain, others impregnated with starch. Some dishes were filled with normal and others with irradiated petrolatum. After several hours at 38° C., the sections of film were removed and the gelatin scraped off each into tubes of cold water. A blue color was developed in the tubes of the starch emulsion from the dishes of irradiated petrolatum when an acidified iodide solution was added. The scrapings of plain gelatin were red after Schiff's reagent had been added to these tubes. The control reactions with gelatin exposed over normal petrolatum were negative with both reagents. The Schiff reagent used gave negative reactions with perhydrol.

The previous experiments permit the conclusion that the photoactivity of irradiated petrolatum and mineral oil is chemical, that a substance is activated by ultraviolet light in the presence of oxygen which vaporizes readily, that organic peroxides and aldehydes are formed, and that these aldehydes and peroxides diffuse readily through a film of acetocellulose.

#### BACTERICIDAL EFFECTS

Although many essential oils are normally bactericidal, an effect occurring chiefly with those containing alcohols or aldehydes, the effect of the vapors has been little studied. Experiments in this direction are difficult because vaporization occurs so readily that bacteria exposed to the vapor are soon smothered in a coating of oil. Hence, a comparison of the bactericidal effects, before and after irradiation, of the oils or the vapors of oils which normally kill bacteria is unsatisfactorily inconclusive. But Wrenn,<sup>7</sup> in 1927, observed bactericidal action with vapors of irradiated oils which were not bactericides in the un-

irradiated state With inconclusive evidence he attributed this activity to light emanations Harris, Bunker and Milas, just recently, after an extensive study of the bactericidal effects of vaporous emanations from numerous irradiated vegetable and animal oils, concluded that the bactericidal effects were due to peroxides They found a direct correlation, not between the initial or final peroxidic oxygen content of irradiated oils, but between the increase in peroxidic oxygen content occurring during irradiation, and the germicidal effect of their vapors

Investigations of the bactericidal effects of irradiated petrolatum and mineral oil have been made Ross<sup>5</sup> reported that *Staphylococcus aureus*, but not *Bacillus pyocyaneus*, was killed when a loop of these bacteria from agar was emulsified in an irradiated mixture of petrolatum and lanolin Thompson and Sheard repeated these experiments They made bactericidal counts from hour to hour from the emulsion of bacteria and irradiated petrolatum and compared these counts with counts of bacteria suspended in normal petrolatum The counts on the irradiated mixture showed a steady and rapid decrease in bacteria A decrease was also encountered in the unirradiated mixture Harris, Bunker and Milas exposed films of bacteria on agar plates to the vapor over irradiated mineral oil The bacteria were not killed These *in vitro* experiments, however, fail to furnish the conditions under which a clinical germicide must act Sears and Black<sup>6</sup> have recently carried out *in vitro* experiments, simulating the conditions under which a bactericidal oil must act *in vivo* The anaerobes studied were not affected, nor were bacilli of the colon typhoid group killed by irradiated petrolatum *Staphylococcus* and *Streptococcus*, both viridans and hemolyticus, were not viable after prolonged contact with the irradiated oil These authors conclude that the mild bactericidal effect observed is due to a nonvolatile compound formed under the oxidative action of ultraviolet light

To exactly simulate *in vitro* the conditions encountered in an infected wound is impossible The nearest approach is an experiment in which the bacteria are suspended in minute globules of nutrient media emulsified in the oil or petrolatum In preliminary experiments freshly spread films of several aerobic pathogens on blood agar plates were exposed twenty-four hours in the incubator to the vapor over irradiated petrolatum Several common anaerobes were similarly exposed during incubation in Fildes jars Next, 1 cc of liquid culture of each of these bacteria was centrifuged, and the residual bacterial sediment thoroughly mixed with 5 cc of warmed irradiated petrolatum Cultures were made in twenty-four hours In the third experiment 1 cc of uncentrifuged liquid culture of each of these bacteria was emulsified in irradiated mixtures of mineral oil and petrolatum of a consistency in which a fine suspension of the culture was possible After twenty-four hours the tubes were warmed, the media centrifuged to the bottoms of the tubes and cultured Finally, 1 cc of fresh medium was inoculated with a loop of the growing cultures Irradiated mixtures of warmed mineral oil and petrolatum were added and the liquid media were emulsified finely in the oil The tubes were incubated aerobically or anaerobically according to the requirements of the bacteria After four days these tubes were warmed and centrifuged Films and cultures of the sub

jacent media showed luxuriant growth. The aerobes studied were *Staphylococcus aureus*, a virulent strain of *Streptococcus hemolyticus*, *Neisseria catarrhalis*, *Streptococcus viridans*, a virulent *Streptococcus pneumoniae*, *Hemophilus influenzae*, *Pasteurella lepi-septica*, and *Bacterium coli*. The following Clostridia; chauvaci, histolyticum, novyi, oedematiens, sepique, sporogenes, and welchii, were subjected to the above experiments. None was killed nor was growth apparently inhibited.

#### DISCUSSION

The experiments undertaken show conclusively that, as a result of oxidation occurring during exposure of vaseline or liquid petrolatum to ultraviolet light, vaporous peroxides and aldehydes are evolved. Neither the oils nor the vapor from them were sufficiently bactericidal that a germicidal effect on strains of virulent aerobes or stock strains of anaerobes could be demonstrated in vitro. Other oils, according to some authors, when irradiated acquire definite bactericidal properties. They correlate this bactericidal effect with the increase in peroxidic oxygen occurring under irradiation.

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## BACTERICIDAL ACTION OF SHORT AND ULTRASHORT WAVES\*

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THE work of foreign authors has shown a specific bactericidal action of ultra short waves. This means that the electronic action of these ultrahigh frequencies has a lethal action on bacteria because of the peculiarities of the frequency itself and not because of any other effects as induced heat. Furthermore, different bacteria respond differently to different wave lengths, a four meter wave, for example, may not kill a bacteria, the destruction of which may, however, be accomplished by a 15 meter wave. In short, the object of this paper is to determine the specific action of ultrashort waves on bacteria.

*Experiment I*—Four bouillon culture tubes of *Staph aureus* were used. Each tube contained 5 cc. of culture. The tubes were then placed in a glass beaker filled with ordinary tap water. Since the dielectric constant and conductivity of the water is less than that of the bouillon, the tap water would be exerting no shielding action. A clear bouillon tube was also added, and its temperature was taken to indicate the temperature rise of the bouillon cultures. The condenser plates in all these experiments were put one inch from sides of the glass beaker. Ten meter wave --- 200 watts. Temperature of bouillon cultures and tap water at start was 33° C. As the temperature of the water began to rise part was drained off and cold water to the same amount added. In this way the temperature of both the water and bouillon did not vary more than 2° C. during the course of the exposure. This means of controlling the temperature rise was used in all the experiments.

Tube 1	Exposed fifteen minutes
Tube 2	Exposed thirty minutes
Tube 3	Exposed forty five minutes
Tube 4	Exposed sixty minutes

Subcultures were then made and active growth noted. On the following day Tube 4 was exposed sixty minutes subcultures were made and active growth was noted. On the following day, Tube 4 was exposed sixty minutes and active growth was noted. Tube 4 of *Staph aureus* had therefore three hours of exposure on three successive days and no effects were noted.

*Experiment II*—The above experiment was done with streptococcus in chains, ten meters 200 watts. Tube 4 of the streptococcus, after a total of three hours of exposure on three successive days, showed active growth.

*Experiment III*—The above was done with typhoid bacilli, 10 meters, 200 watts. Tube 4 after three hours of exposure on three successive days showed active growth.

*Experiment IV*—The above was done with pneumococcus, 10 meters, 200 watts. Tube 4 after three hours of exposure on three successive days showed active growth.

*Experiment V*—The above four cultures were exposed on three successive days for a total of three hours to 15 meters, 200 watts. Subcultures showed active growth in all four.

*Experiment VI*—The above four cultures were exposed for three hours on three successive days to 6 meters, 185 watts. Subcultures again showed active growth.

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Acknowledgment is made to the Elmer and Amend Company for their kind cooperation in the above experiments.

*Experiment VII.*—The above four cultures were exposed to 6 meters, 300 watts for a total of two hours on two successive days. Subcultures again showed active growth.

From the above experiments we can say that short and ultrashort waves in the ranges indicated have no specific action on bacteria in bouillon cultures.

*Experiment VIII.*—Agar plates having cultures of *Staph. aureus* and *Streptococcus* in chains were now put in the condenser field. It was important here to prevent heating of the plates above the lethal temperature of the bacteria. A precision thermometer was placed between two agar plates in the field and in this way the temperature of the plates was found. This of course did not give the absolute temperature of the agar cultures but sufficed to help in keeping the temperature below the lethal point of the bacteria. When the thermometer read 37° C., the agar plates were removed from the field, cooled and then exposed again. In this way the plates were exposed for a total of two hours. They were exposed to 10 meters at 200 watts, and 15 meters at 200 watts. Subcultures were then made and active growth noted.

Our experiments failed to show a bactericidal action of short and ultrashort waves, limited to the respective wave lengths and power used. Of course the body is not a test tube, and in the body certain secondary effects from the use of short waves may have a decided influence upon bacterial life.

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EDWARD PODOLSKY, M.D., BROOKLYN, N. Y.

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A great many physicians have gained fame in literature, perhaps more than in any other calling, although it is interesting to recall that Richard Jordan Gatling, inventor of the Gatling gun, David Livingstone, who penetrated the depths of Africa, Leander Starr Jameson of the infamous or famous Jameson Raid into Transvaal, Leonard Wood, soldier and ruler of provinces, and Theodore Newton Vail of telephone fame, were all physicians.

Rabelais, immortal author of the classics *Pantagruel* and *Gargantua*, was born in 1490, and studied medicine at the University of Montpellier. In 1530, he received his Doctor of Medicine. For many years he was a busy practitioner

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Rabelais' stories are notable because they are a mixture of insane laughter and mock gravity, because they point out the childishness and grandeur of the commonplace. Rabelais was the first great laughier in literature. He saw life in all its sordid details, and he could not be serious about it.

Among the most notable litterateurs in the dawn of German literature was Friedrich von Schiller, who was born in 1759, and whose plays, *The Robbers*, *William Tell*, *Marie Stuart*, *Maid of Athens*, are among the greatest in the German language. He was graduated in medicine, and for several years was a military surgeon. His success in literature, however, caused him to abandon his medical practice in order to devote more time to his novels and dramas.

One of the greatest poets in any language was John Keats. Few people know that Keats was a qualified physician. He was born in 1795 and during his youth became apprenticed to Thomas Hammond, a prominent surgeon in Edmonton. For some reason or other, he could not get along with Hammond and broke off his apprenticeship. He, however, continued with his medical studies and became a dresser at Guy's Hospital. While at the hospital Keats made the acquaintance of Leigh Hunt, Benjamin Haydon, and Shelley, whose company and ideals he found more congenial than those of his medical associates. It was in 1816 that Keats definitely abandoned medicine, for which he had little taste, two years later he published *Endymion*, which won him imperishable fame.

Dr. John Brown, born in 1810, was not only a Scotch doctor with a large practice, but a talented man in many directions. He was graduated in medicine from Edinburgh and enjoyed practice among his people. He belongs to that group of authors who become known as "one book" authors, that is, their fame rests wholly on but one book. Dr. Brown is immortalized in literature as the author of the greatest of all dog stories—*Rab and His Friends*.

Oliver Goldsmith, one of the glories of English literature, author of the classics *The Deserted Village* and *The Vicar of Wakefield*, was duly graduated as a doctor and even wrote two medical books. He was of unstable disposition and constantly getting in and out of difficult situations. He tried not only medicine as a means of gaining a livelihood, but also several other professions, and in none was he successful. At length he determined to try his fortune in America. While at a convivial gathering he enjoyed himself more than was wise for him, and his ship sailed without him.

He then decided to study law for which purpose he borrowed fifty pounds. He never realized this ambition because he promptly lost his fifty pounds at a gambling table. At the age of twenty-four, somewhat sobered, he went to Edinburgh to study medicine. For some strange reason or other he was able to get through his courses. But it was not long before his old restlessness returned. With a flute for his companion, he vagabonded over Flanders, France, and Switzerland. He returned at length to England to settle down, and for awhile he eked out a meager existence by pounding drugs and running errands for apothecaries. A little later he was successful in securing a medical appointment with the East India Company, but it was revoked. He also took examinations for a hospital



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much superior to his Sherlock Holmes stories have never been as popular as they deserve to be. Even more popular were his scientific stories featuring Professor Challenger. Professor Challenger, like Sherlock Holmes, had his counterpart in real life. He was Professor William Rutherford, the physiologist. For his *History of the Boer War* he was knighted. When he lost a son in the World War his interest turned to spiritualism which resulted in a steady stream of spiritualistic books from his pen.

Silas Wen Mitchell was another of the popular American novelists who was also a great doctor. He wrote voluminously on both medical and popular topics. In medicine he became known as the originator of the "rest cure" and in popular literature he gained fame as the author of a very successful novel, *Hugh Wynne*.

Russia produced one of the most glorious of the doctor authors, one of the greatest of short story writers, Anton Chekhov. Chekhov practiced medicine during the early part of his adult life and was rather successful, but tuberculosis caused him to curtail his medical activities. He was already making a name for himself as a writer and dramatist. In his short existence he produced some of the greatest stories and dramas which even today are produced throughout the world with success.

John McCree is a poet whose fame will rest on one great poem he wrote, perhaps the greatest produced by the World War. He was born at Guelph, Ontario, Canada and studied medicine at Toronto. In 1899, he went to McGill University to assume his duties as a pathologist, a little later becoming a lecturer in clinical medicine. In October, 1914, he went overseas as a medical officer with the first Canadian contingent. Four years later he was appointed a consulting physician to the British armies in the field, but before he could enter on his new duties he contracted double pneumonia from which he died. His great poem, *In Flanders Fields*, was published in *Punch* on December 8, 1915.

The late Poet Laureate of England, Dr. Robert Bridges, was a physician who wrote some excellent medical papers before he turned to writing poetry. He left behind some of the greatest of recent English poems. Dr. Henry Drummond, popularly designated, though unofficially, the Canadian Poet Laureate, practiced medicine in Montreal and elsewhere in Canada for more than twenty years before he finally gave up his practice to describe the life and character of French habitant life in his poems, which have attained a very wide vogue.

The doctor has contributed and is contributing to current literature with greater glory than ever before. The contemporary doctor author is a very important part of living literature. Perhaps the greatest of living physician writers is Somerset Maugham, whose plays and novels are now among the classics. Maugham was born in Paris in 1874, the son of the attorney to the British Embassy. He received his education at King's College, Cambridge, and the University of Heidelberg. It was his intention to become a painter, but when his family returned to England he seemed to have lost this ambition and instead determined to study medicine. He received his medical degree but never practiced. His first novel, *Liza of Lambeth*, was based on his experiences in the hospital in which he seemed his medical education, but the novel didn't make

much of an impression. This should perhaps have discouraged Maugham from using his own experiences as the basis of a novel, but it didn't. He felt that he had an interesting story to tell, the story of a medical student with a physical handicap, and what life meant to him. He told his story in *Of Human Bondage*, and this proved to be the greatest novel he ever wrote, and a work of fiction which became a classic. He later turned to play writing, in which field he became as popular as in the writing of novels.

Francis Brett Young is another of the older English physician-authors whose greatest novel *My Brother Jonathan* like *Of Human Bondage* deals with medical life. *Portrait of Clare* and *Mr. and Mrs. Pennington* are two other novels of Dr. Young which have proved very popular. Another author belonging to the same group is Warwick Deeping whose greatest novel, *Sorrell and Son*, also deals with medical life. Dr. Deeping is the son and grandson of physicians. He received his medical training at Cambridge but was more interested in literature than in medicine. During the War he served in Gallipoli and France in the Royal Army Medical Corps. After the War Dr. Deeping devoted himself exclusively to fiction writing, producing *The Ten Commandments*, *Stories of Love and Courage*, *Ropers Row* and others.

Within very recent years another doctor has joined the group of English doctor-novelists. He is Archibald Joseph Cronin, whose first novel, *Hatter's Castle*, became a world best seller. A second novel, *Three Loves*, although not as popular, also attained world-wide fame. Dr. Cronin was born in Cardross, Scotland, and received his Doctor's degree at Glasgow. He served during the War in the medical corps and later did medical research in Wales for the Department of Mines. After a very busy practice in London his health became undermined and he turned to writing as a recreation. He was so successful as a novelist that he determined to devote all his time to writing.

One woman physician has attained a reputation as a writer with a novel *Doctor Serocold*. She is Dr. Helen Johnson who has written under the name of Helen Asthon. She received her medical training at London University, but after her marriage she retired from practice to devote herself to writing. Besides *Doctor Serocold* she has written *Far Enough* and *A Background for Caroline*.

The best writers of detective stories have been physicians. When Sir Arthur Conan Doyle died his mantle descended upon the shoulders of Dr. Richard Austin Freeman, creator of Dr. Thorndyke, whose adventures are known to millions. Many believe that Dr. Freeman is the greatest of modern detective story writers. At eighteen he began the study of medicine at Middlesex College, and only six years later was made a Fellow of the Royal College of Surgeons. After an attack of tropical fever contracted while in Africa, he returned to England to resume his practice, but his health, always very poor, did not permit it. He turned to writing and particularly to creating detective mysteries which have carried his name throughout the world.

Among American authors there are several who deserve mention. Dr. John Rathbone Oliver, both priest and doctor, has written some of the best

novels of today His *Victim and Victor* and *Fear* were best sellers Dr Oliver leads a very satisfying life He is a psychiatrist, a priest, a professor of medical history at Johns Hopkins University, and a novelist of great power

A unique American author doctor, Charles Alexander Eastman, a full blooded Indian, has written some of the best stories of American Indian life His books, *Smoky Days*, *Wigwam Evenings*, *The Red Hunter* and *The Animal People*, have enjoyed a world wide popularity

Among other American physicians who have attained fame as writers are Charles Conrad Abbott, J A Markev, Alden Arthur Knipe, James Bayard Clark, C E Blanchard, Edward C L Adams, and William Carlo Williams Edward C L Adams has written some of the best stories of American negro life Among his best known stories are *Congaree Sketches*, *Nigger to Nigger*, and *Potee's Gal* William Carlo Williams is known for his two novels *In the American Grain* and *Voyage to Paganry*

The glorious tradition of the physician author is still being carried on by an ever increasing number of physicians throughout the world In Germany it is Alfred Döblin, author of the world success, *Alexander Platz*, in the United States, Robert McNair Wilson, William H Holcombe, Arthur Gash and others, in the Philippine Islands it is Jose Rizal, whose *The Eagle's Flight* has been compared in power to *Uncle Tom's Cabin* The doctor has been among the most significant makers of literature from the earliest times He still is

7110 NINETEENTH AVENUE

## BLOOD SEDIMENTATION RATE IN DIABETES MELLITUS\*

AN ANALYTICAL STUDY OF 510 TESTS PERFORMED ON 366 PATIENTS

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THE blood sedimentation test has been used in various conditions, particularly in tuberculosis and pelvic inflammatory disease Its value as a guide in determining the degree of activity of an inflammatory process has been more or less accepted

The question as to whether this test could be applied in studying diabetes was considered, first, because of the prevalence of low grade infections in this disease, second, because we wanted to investigate whether diabetes per se had any influence upon blood sedimentation

Referring to the first problem we thought it might be of interest to learn whether the commonly accepted focal infections have any systemic influence upon the diabetic individual, employing the blood sedimentation test as a guide

The second problem was to learn if possible, what influence diabetes has

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upon the sedimentation rate. Having in mind the tendency of diabetic patients to develop infections because of their lowered resistance, we were curious to know whether or not this tendency could be recognized by studying the index of sedimentation upon a group of diabetics.

*Method of Study and Technic.*—Five hundred and ten tests were made upon 366 patients. Office and out-patient cases embraced this series. They were not selected. A record was made of the duration of the disease, the age of the patient, and the presence of focal infections: such as the condition of the teeth, the tonsils, evidence of sinus infection, gallbladder disease; notation was also made of the presence of other infections such as arthritis, pyelitis, pulmonary diseases, cellulitis and any other conditions which may have some

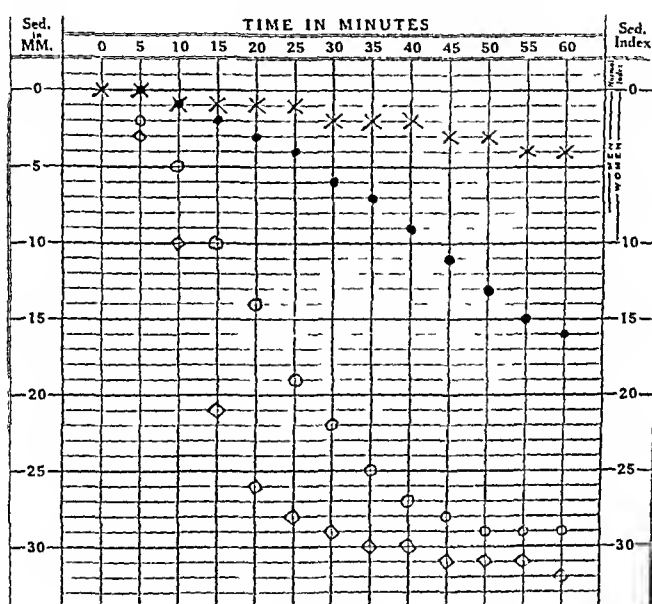


Fig. 1.

influence upon the blood sedimentation. Blood sugar estimations were made at the same time that the blood was obtained for the sedimentation test. Data obtained from this series of tests and observations were recorded and arranged in tabular form. These tables will be discussed later.

The *technic* employed in this study was the one advocated by Cutler.<sup>1, 2</sup> Time and space do not permit a discussion of the various tests employed for blood sedimentation. Blood was obtained by venapuncture. When working with the 5 c.c. tubes, 4.5 c.c. of blood were added to 0.5 c.c. of 3 per cent sodium citrate or if the 1 c.c. tubes are in use, add 0.9 c.c. blood to 0.1 c.c. of the citrate solution. Either test may be employed. The tubes containing the mixture are shaken gently, and then permitted to stand for sixty minutes. Readings are taken every five minutes and the level of the cells is recorded on graphs, graded in millimeters. At the end of the sixty-minute period, the graph may be interpreted.

The resulting curves or lines are classified according to the rate and degree of sedimentation. These may be grouped in the following types:

1. The *horizontal line* drops down slightly but remains within the normal range of 0 to 8 mm in males, 0 to 10 mm in females. This is the graph one sees in normals or where the pathologic process, if any exists, is inactive.

2. *Diagonal line* in which the sedimentation seems to occur at regular intervals forming a straight line from 0 to the final reading, which is usually between 10 and 20, but may extend lower.

3. *Diagonal curve* drops faster than the diagonal line, then settles down to a slower period of what is described as "packing." This graph is usually met with when the sedimentation index ranges from 20 to 30 mm.

4. *Vertical curve* where the red cells settle more quickly the line dropping rapidly, usually reaching a drop of 20 to 25 mm within the first half hour and then the graph develops a curve of "packing." This type of graph is seen in the acute and more active infections.

For illustration, the above mentioned types are depicted on the sedimentation chart in Fig. 1.

*Diabetes and Blood Sedimentation*—Of the 510 tests 164 were of the horizontal line group, 262 were diagonal lines, 76 were diagonal curves, and 8 were vertical curves. The combined abnormal curves were 346 or 67.8 per cent. The horizontal lines were about one third or 32.2 per cent.

A more detailed study was made of the records. They were arranged according to the type of curve. These different groups were analyzed and figures obtained according to the duration of the diabetes, to the blood sugar, and the age in decades. Results may be seen in Tables I to III.

TABLE I

ANALYSIS OF THE BLOOD SEDIMENTATION RATE ACCORDING TO THE DURATION OF DIABETES

DURATION	TOTAL CASES	HORIZONTAL		ABNORMAL	
		NO.	PER CENT	NO.	PER CENT
Up to 3 yr.	192	66	34.4	126	65.6
3 to 5	104	37	35.6	67	64.4
6 to 10	123	38	30.9	85	69.1
11 up	86	25	29.1	61	70.9
Not mentioned	5	—	—	5	100

The percentages in both groups seem to vary only slightly. The difference between the early diabetic patients (34.4 per cent) and those who have had diabetes eleven years or more (29.1 per cent) is only 5.3 per cent. This difference does not seem significant enough to indicate any influence of the duration of the disease upon the sedimentation rate, because one can surely attribute this disparity in figures to age and the possibility of infections developing in older people.

The next consideration of the diabetes sedimentation problem was the study of blood sugar estimation at the time when the sedimentation tests were performed. The results are arranged in Table II.

If we separate the above records into Group A, embracing the sedimentation rates of those who had a blood sugar of 180 mg. or below, we find that



69 cases or 31.2 per cent showed horizontal lines and 152 records or 68.8 per cent had abnormal ones. Comparing this with the hyperglycemia Group B, we find that 95 showed horizontal lines (32.9 per cent) and 194 (67.1 per cent) had abnormal lines. Another interesting fact: if we single out for illustration, the 15 mm. diagonal line series which showed the largest individual total

TABLE II

SHOWING THE BLOOD SUGAR IN MG. PER 100 C.C. COMPARED WITH THE INDEX OF SEDIMENTATION

BLOOD SUGAR MG.	HORIZONTAL NORMAL	DIAGONAL LINE			DIAGONAL CURVE ABNORMAL 15-30	VERTICAL CURVE 25-35	TOTAL ABNORMAL
		15	20	25-30			
-120	20	23	16	4	8	—	51
121-150	35	22	16	1	8	2	49
151-180	14	15	12	6	17	2	52
Total: below 180	69	60	44	11	33	4	152
181-200	25	13	18	4	5	2	42
201-250	34	26	28	5	6	—	65
251-up	36	24	21	8	32	2	87
Total: above 180	95	63	67	17	43	4	194

of 123 records, in 60 of these the blood sugar was 180 mg. or below, while 63 belonged to the hyperglycemic group. One is impressed with the similarity of the percentages in both groups. The difference of 1.7 per cent is striking and seems to be a convincing argument against the effect of the blood sugar upon the sedimentation rate. This deduction concurs with the conclusions of Wisselink,<sup>3</sup> Remer,<sup>4</sup> Goedel and Hubert.<sup>5</sup>

*Age.*—An analysis of the sedimentation curves in reference to age was studied and arranged in Table III.

TABLE III

SHOWING AN ANALYSIS OF THE BLOOD SEDIMENTATION GROUPS ACCORDING TO AGE IN DECADES

AGE	HORI- ZONTAL		TOTAL	DIAGONAL LINE				DIAGONAL CURVE				VERTICAL CURVE			TOTAL ABNOR- MAL	COMB. TOTAL
	5	10		15	20	25	30	15	20	25	30	25	30	35		
to 20	7	3	10	2	2	--	--	--	--	--	--	--	--	--	4	14
21-30	7	9	16	6	3	--	--	--	--	--	--	--	--	--	9	25
31-40	5	8	13	11	9	2	--	1	--	--	--	--	--	--	23	36
41-50	15	39	54	32	33	5	--	--	6	10	1	--	1	--	88	142
51-60	5	31	36	44	30	13	--	--	8	16	7	1	--	3	122	158
61-up	10	25	35	28	34	6	2	1	4	15	7	1	2	--	100	135
Total	49	115	164	123	111	26	2	2	18	41	15	2	3	3	346	510
	164			262				76				8				

We noticed that the younger patients coming under our observation showed either horizontal lines or the slightly abnormal curves. Since diabetes is a disease which occurs more commonly in the fifth, sixth, and seventh decades, we naturally would expect to find the greater number of records in the older diabetic patients. However, it was interesting to observe that the abnormal rates were found in greater numbers proportionately with the in-

crease in the age groups. We are not prepared to offer the exact cause of this observation, but we presume that the reasons for the abnormal sedimentation rates in the aged are most likely due to cell destruction resulting from wear and tear of the tissues and the fact that the aged are more liable to develop infections which may exert some influence upon the rate of sedimentation.

*Infections*—An analytical study was made of the various records for the presence of infections. The results are arranged in Table IV.

TABLE IV  
SHOWING THE PREVALENCE OF INFECTIONS IN THE VARIOUS SEDIMENTATION GROUPS

	HORIZONTAL		DIAGONAL LINE				DIAGONAL CURVE				VERTICAL (CURVE)			TOTAL
	5	10	15	20	25	30	17	20	25	30	25	30	35	
Teeth														
Good	16	14	16	3					1					50
Bad, pyorrhea	2	12	12	1	7		1	9	2		1	1	2	64
Alveolar abscess			1	1										2
Missing	13	53	16	61	16		10	27	12		12			200
Tonsils														
Enlarged	7	28	13	21	6		3	6	3		1			90
Diseased	22	60	32	61	15		7	17	3		1	2	1	241
Pharyngitis	20	32	33	23	4		1	15	1					129
Sinusitis	3	8	13	18	1		2		1		1			40
Colds (upper respiratory)			6	5	1		1	1	1		1			16
Tuberculosis														
Clinical	1		4	10	1				2					18
Proved									1	1				2
G B														
Suggestive		6	12	16	1		1	4	1					41
Definite	2	5	2	6	2		1	2						24
Renal pyelitis, cystitis	1	8	6	1					1	2		2		21
Cellulitis and boils		5	3	4					3	2				17
Arthritis and osteo arthritis	1	9	10	6					4	2		1		36

One meets with obstacles when attempting to interpret the results of the sedimentation tests in the presence of minor infections. Sufficient work has not been done along these lines to furnish us with guides. Then again, the presence of combinations of focal infections will prevent us from definitely isolating the chief offender.

It is not our intention to become involved in a discussion of infections in diabetes. We were chiefly interested to learn whether the sedimentation will drop to abnormal ranges in patients with bad teeth, diseased tonsils, sinus conditions, etc.

In this table one may notice that a vast majority of the patients with bad teeth are in the abnormal groups. Most of the *pyorrhea* cases behaved similarly. However, a fairly high percentage of the patients with good teeth showed abnormal lines. We must bear in mind what was mentioned above, namely, that there may be other forces besides the teeth which may affect the sedimentation rate in these patients.

In the *tonsil* series, it was noticed that about one-third of the records of patients with diseased tonsils showed horizontal lines. In the remaining cases we found abnormal drops in sedimentation. It may be possible that the absorption from the diseased tonsils had some effect upon blood sedimentation.

The question of the effect of *sinus* infection holds a similar position as the tonsils. Of the 49 records of patients with a history of sinus disease, 13 showed horizontal lines; the balance of 36 had abnormal lines (73.4 per cent). While in this group we may consider those patients with upper respiratory *colds*: 16, the entire number, showed abnormal lines, 11 of them belonging to the milder diagonal group.

In the pulmonary diseases, the *tuberculosis* series, 19 of the 20 had abnormal lines. This phase of the subject need not be discussed because many contributions have been published on the clinical value of blood sedimentation in tuberculosis.

*Gallbladder* disease showed a vast majority in the abnormal ranges.

In *pyelitis* and *cystitis*, abnormal lines were present in slightly over 50 per cent.

In the series of *arthritis* and *osteoarthritis*, 26 of 36 tests showed abnormal lines. However, one must keep in mind the presence of focal infections in these patients and their possible effect upon the sedimentation rate.

#### SUMMARY AND CONCLUSIONS

A study of 510 sedimentation tests was performed upon a group of 366 patients; 346, or 67.8 per cent, showed abnormal readings.

The explanation for this high percentage must be either the diabetes or the presence of infections, or both.

A study of the duration of the diabetes showed that the maximum difference of the percentages in the various diabetic groups was only 5.3 per cent. These figures evidently do not permit us to attach much significance to the effect of the duration of the diabetes upon the sedimentation rate.

The possible influence of the blood sugar was also studied. There was a striking similarity of the percentages in the hyperglycemic group and those whose blood sugar was 180 mg. or below: in the former there were 194 cases (67.1 per cent) which showed abnormal lines, and in the latter (nonhyperglycemic group), there were 152 records (68.8 per cent) with abnormal lines. From our studies we concluded that the blood sugar per se had no influence upon the sedimentation rate.

Since the duration of the diabetes and the blood sugar cannot explain the high incidence of the abnormal sedimentation rates, the inference is that the most likely explanation is infection. Analysis of the patients observed in this study showed a high incidence of the minor infections, particularly in the teeth, tonsils, and upper respiratory tract. However, the urinary tract and gallbladder must also be kept in mind. The effect of tuberculosis upon sedimentation does not require any comment here.

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## SUBCLINICAL SCURVY IN CHILDREN\*

## THE APPLICATION OF THE CAPILLARY RESISTANCE TEST

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WITH THE TECHNICAL ASSISTANCE OF RICHARD F COUSINS

AT THE request of the late Dr Alfred F Hess of New York City, a study was undertaken in the New Jersey State Home for Boys to determine the specificity of the capillary resistance test for scurvy. He considered the diet of our boys adequate in all respects, especially the intake of antiscorbutic foods.

Each boy receives about one quart of milk daily in some form or other. Vegetables are supplied daily from our own farm and truck garden. During the winter months the surplus of vegetables which have either been stored or canned is served the boys. In addition butter, cereals, and meat are part of the daily diet. Tomatoes are in abundance while citrus fruits are less evident.

The capillary resistance test was first used by Rumpel<sup>1</sup> in 1909 for diagnosing scarlet fever without a rash by means of a Bier compression band placed about the upper arm thereby producing hemorrhages in the skin. Leede<sup>2</sup> in 1911 further elaborated this test using the Riva-Rocce apparatus. Hess and Fish<sup>3</sup> in 1914 further improved the test and were the first to use a blood pressure tourniquet. Gothlin<sup>4</sup> after several years of experimentation modified and improved the method, we followed his technique in detail without attempting any modification on our part. Dalldorf<sup>5</sup> improved a method of applying negative pressure to the surfaces of the skin through a suction cup connected with a mercury manometer and vacuum pump.

The purpose of the capillary resistance test is to determine the ability of the small blood vessels to withstand increased intravascular pressure which is brought about by means of a tourniquet applied to the arm. In 1920, Hess<sup>6</sup> in his classic treatise on scurvy stated that the test is not specific for this disorder but that it is positive in the majority of cases of scurvy. It has been generally accepted that capillary fragility, leading to the appearance of petechiae, is one of the earliest detectable manifestations of the effects of a scurvy producing diet in

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animals. Hess<sup>7</sup> in 1921 suggested that a "failure of the integrity of the epithelium of the blood vessels" occurred and that "this was due to a lesion of the endothelial cells or their cement substance." Wolbach and Howe<sup>8</sup> believed that the defect in the capillaries results from a failure of the endothelial cells to form cement substance and they concluded that the essential pathologic alteration in scurvy is "an inability of the supporting tissue to produce and maintain intercellular substances."

Hess<sup>9</sup> further questions the specificity of the test by stating, "in my experience there are decided individual variations in regard to the reaction to this test, so that although it is true that petechial spots are far more numerous in individuals suffering from latent or active scurvy, the reaction cannot be used as evidence of a deficiency in vitamin C intake. Occasionally edema rather than hemorrhage has been found in the scurvy of adults and of infants. The cause of these extravasations, which have been noted most often at the ankles, is not known. It would seem most likely that it is associated with a retention of sodium chloride." Clinical studies of this aspect were published by Meyer<sup>10</sup> in 1923, by György<sup>11</sup> in 1927, by Öhnell<sup>12</sup> in 1928, and by Göthlin<sup>13</sup> in 1931. Öhnell first suggested the use of the test in the recognition of mild forms of vitamin C deficiency.

The method used by us was identical with that developed by Göthlin<sup>4</sup> and the reader is referred to his article for details and procedure. An excellent photograph of the apparatus attached to the patient and ready for use is also found in the above article. Our boys were all tested in the afternoon and in the horizontal position. The arms were in the same plane as the heart to minimize differences of pressure. The room temperature was comfortable and more or less controlled to prevent chilling. The light under which the readings were made was standardized by using an electric lamp with the same size bulb and reflector for all the tests.

A colored ring, of 60 mm. diameter, was imprinted with a rubber stamp on both arms with its center coinciding with the center of the hollow of the elbow. The area of the skin thus outlined was thoroughly inspected through a magnification lens (5D) before pressure was exerted. If any formations which might have later been mistaken for fresh petechiae were visible they were marked for exclusion. The rubber tourniquet was then wound about the arm and fixed, the lower edge of the tourniquet being at least 2.5 cm. above the nearest part of the colored ring. This is important as occasionally petechiae appear in greater number just below the lower edge of the tourniquet. The pressure in the tourniquet is quickly raised to 50 mm. of mercury and kept there by means of a screw compressor. In our work three boys were done at the same time with one mercury manometer. A blood pressure bulb was used to pump the air in and the screw compressor was used to make the finer adjustments. During the fifteen minutes that the pressure was maintained, we found it necessary to make slight adjustments not more than twice during the period. The petechiae were counted within fifteen minutes after the tourniquet was removed and the average of both arms recorded. An average of more than 8 petechiae was con-

sidered "positive" while those between 5 and 8 were called "transitional" and those below 5 were considered "negative"

Gothlin has gone into this subject rather extensively and is convinced that the capillary resistance test is specific for scurvy. He<sup>1</sup> placed two healthy adult demented patients on a scorbutic diet for a few weeks and found that the original normal strength of their skin capillaries fell to an abnormally low level but that this change in the capillary strength was reversible, the strength returning gradually to normal when a sufficient amount of orange juice was given for several weeks. Gothlin mentions that positive results may be obtained in acute infectious diseases and in cases of albuminuria. Thus we have confirmed and we have also obtained positive results in one boy who has chronic asthma (sensitive to ragweed pollen) and in one boy who had recently recovered from a dermatitis venenata (poison ivy). One boy with ichthyosis of the skin gave a negative result but was not included in our data because we were not sure of our readings.

Our tests were all done between January and May, 1934, the time of the year when the stored amount of antiscorbutic vitamin is probably at its minimum. The boys were free of any acute infections and all of our positive cases were examined in more detail to eliminate any abnormality which might give false positives. Only one boy was found to have a mild gingivitis. Falk et al.<sup>14</sup> found that when the capillary test revealed definite subnormality in the capillary strength and the vitamin C standard this subnormality was in 70 per cent accompanied by gingivitis, and on the other hand that 10 per cent of those children who were examined and found to have a normal capillary strength, and thus a normal vitamin C standard, nevertheless, exhibited mild gingivitic alteration.

There were 969 tests done on 418 boys as illustrated in Table I. Only averages of the number of petechiae found on both arms are reported. Of the 418

TABLE I

969 TESTS WERE DONE ON 418 BOYS. THE GROUP IS DIVIDED INTO THE LIGHT SKINNED NORDIC TYPE (L), DARK SKINNED SOUTHERN EUROPEAN (D) AND THE INTERMEDIATE COMPLEXIONS (I) COMMONLY FOUND AMONG THE AMERICANS

AGE	NUMBER OF CASES			NEGATIVES (14 PETECHIAE)			TRANSITIONALS (5-8 PETECHIAE)			POSITIVES (ABOVE 8 PETECHIAE)		
	L	I	D	L	I	D	L	I	D	L	I	D
9		1			1							
10	6	1	1	4	1	1	1			1		
11	4	6	5	4	5	4					1	1
12	6	20	3	5	16	3	1	2			2	
13	4	24	7	3	20	7	1	2			2	
14	11	39	13	9	34	11	1	4	1	1	1	1
15	17	64	16	14	59	15	2	5		1		1
16	18	79	27	17	74	25		3	2	1	2	
17	4	21	10	3	16	9	1	3			2	1
18	1	5	4	1	5	4						
Totals	72	60	86	61	231	79	7	19	3	4	10	4
Per cent	17.2	62.2	20.6	84.7	88.9	91.9	9.7	7.3	3.5	5.6	3.8	4.6
Grand totals	418			371			29			18		
				88.7%			6.9%			4.3%		

boys, 4.3 per cent were found to be positive, 6.9 per cent transitional, and 88.7 per cent negative.

Many boys, without any treatment, were retested at intervals of one or more months and in almost every instance again fell into their original groups. Seventy-one boys gave zero results on both arms, 19 were retested and all fell again into the negative category, but only 7 of these 19 again tested zero on both arms. Fifteen boys who were found to have an average of more than 8 petechiae (positives) were retested, without previous treatment, and only one boy was found to have fallen into the negative group, while 9 were even more positive than before.

Dr. David Greene of New York City brought to my attention the discrepancies he found when testing both arms of his patients. Göthlin<sup>13</sup> tested both arms on 28 individuals and found that the largest difference was 3 petechiae in any one of them. In a small percentage (3.3) we found 14 boys were positive (more than 8 petechiae) on one arm and negative (less than 4 petechiae) on the other arm. Six of these boys were retested a month later and approximately the same differences were obtained. In 5 boys we found positive results on one arm and transitional (between 5 and 8 petechiae) findings on the other arm. In our group we found that 34 boys or 8.1 per cent had 8 or more petechiae on one arm but only 18 or 4.3 per cent had an average of 8 or more petechiae for both arms. If only the right arms were tested, 28 boys or 6.7 per cent would have been positive, while if only the left arms were tested, 22 boys or 5.2 per cent would have been positive. Therefore our lowest incidence of positives (4.3 per cent) was obtained when the averages of both arms were used. This is just the reverse of what Greene<sup>15</sup> found in a small group of cases. If a small group is being studied the authors recommend that both arms be tested but in very large groups, especially where the presence of scurvy is anticipated, it is suggested that only one arm be tested, preferably the left (Table II).

TABLE II  
COMPARISON OF RESULTS FROM THE USE OF ONE ARM AND OF BOTH ARMS

	AVERAGE OF BOTH ARMS	LEFT ARM ONLY	RIGHT ARM ONLY
% Positive	4.3	5.2	6.7

There appears to be no relation between handedness and the development of petechiae. In our group 21 were left handed (5 per cent) and the remainder right handed. Of the left-handed boys 10 had more petechiae on their right arm, 5 had more on their left arm, while the remainder had equal numbers. Of the right-handed group there was a slight but negligible predominance of the number of petechiae found on the right arm.

Dalldorf<sup>5</sup> believes that the character of the skin itself partly determines the results of the tests. The groups studied included dark-skinned southern Europeans, light-skinned Nordic types, and persons with intermediate complexions, commonly found among Americans. Table I gives the results of this differentiation. The light-skinned group gave a slightly higher incidence of positives, and

there is nothing in our data to support Dalldorf's contention that the character of the skin, in relation to the intensity of pigment, predetermined partly the results of the tests

Hess<sup>9</sup> stated that, 'Economic conditions have, however, always had an effect on the incidence of scurvy, and it is quite possible, if not probable, that the economic depression in this country and abroad may entail an increase in latent and manifest scurvy in adults as well as infants.' Dalldorf<sup>2</sup> recommends that the dietary of the poorer families be studied. Our boys are all committed by the courts of the State of New Jersey because of juvenile delinquency. These boys, in a large percentage come from very poor homes where the diet is irregular and uncertain. Many of these families are "on relief" from the federal, state, or municipal governments. Fifty six boys were tested within a day or two of their admission to the institution. Forty nine were found to be negative, six transitional and one positive. In spite of supposed poor dietary and the presence of dental caries (in almost every boy on admission), the positive results are even less than in our population as a whole. Incidentally in a study made over a period of three years we found that our newly admitted boys were also well nourished. Fifteen of our so called psychopaths who live in a separate unit were tested and all were negative except one transitional.

In an analysis of Table I it is seen that 4.3 per cent were found to be positive. This is an extremely low figure when we compare the data obtained by others. Gothlin<sup>13</sup> found 18 per cent positives among school children between the ages of eleven and fourteen while we found 6.3 per cent positives within this age group. He<sup>14</sup> later studied 3 school groups in different localities and found that 21.5, 5.9 and 19.4 per cent were positives. Where the low percentage was obtained he found that the diet revealed the highest number of guinea pig units of vitamin C. Dalldorf reported from 35 to 66 per cent positives, with the suction method in children from poor homes. Greene<sup>1</sup> in a group of 65 children between the ages of nine and fourteen years found an incidence of 9.2 per cent positives. Within this age range we found an incidence of 6.6 per cent positives. Our low results are most probably due to the adequate diet furnished the boys.

A small group of boys, 5 transitionals and 10 positives were retested at least twice and then treated for three weeks with about 6 ounces of concentrated canned tomato juice daily. Of the positives 2 remained positive 2 became transitional, while the remainder became negative. Of the transitionals all became negative except one who remained in the same category. The above two positives were then given the juice of one orange daily for three weeks and one became negative while the other was tested but not read because of an acute dermatitis venenata (poison ivy). Three additional boys, who had received tomato juice with subsequent negative results were further treated with orange juice with interesting findings: one remained negative while the remaining 2 became positive and more so than before treatment with the tomato juice. The presence of other factors causing this bizarre result was not ascertained. Five boys with positive findings, who had not received tomato juice, were treated with



orange juice for three weeks and all became negative except one who remained positive.

### SUMMARY

There were 969 capillary resistance tests performed on 418 boys to determine the specificity of the test for scurvy and the incidence of positive results before and after treatment with tomato and orange juice. The method employed was the one improved by Göthlin of Upsala, Sweden. Such factors as acute infections, albuminuria, use of both arms, handedness, and color of skin were considered. Boys recently admitted to our institution from poor families were tested to ascertain the adequacy of their diets in relation to antiscorbutic contents.

### CONCLUSIONS

1. The incidence of positive reactions was 4.3 per cent in 418 boys on whom 969 capillary resistance tests were done. This low incidence is probably due to the adequate diet given our boys in the institution.

2. The color of the skin (light Nordic type, dark-skinned southern European type, and the intermediate complexions found among Americans) had practically no relation to the results obtained.

3. There appears to be no relationship between handedness and the development of petechiae on either arm.

4. In testing small groups it is recommended that the averages of both arms be used.

5. Retests, without treatment, proved the test to be consistent in its results.

6. The lowest incidence of positive results was obtained in fifty-six boys who were recently admitted from the "poorer" homes, i.e., from an economic standpoint.

7. A small group of boys, 15 positives and 5 transitionals, were treated with tomato and orange juice and all but 3 cases were benefited.

8. Although we do not consider this test as specific, we nevertheless do think that it is the best available method for the recognition of the subclinical cases of scurvy.

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## EXPERIMENTAL ARTHRITIS IN RABBITS\*

COMPARISON OF THE ARTHRITIS PRODUCING ABILITY OF INAGGLUTINABLE STREPTOCOCCI WHICH RESIST THE "BACTERICIDAL" ACTION OF FRESH, DILUTED, DEFIBRINATED GUINEA PIG BLOOD AND THOSE WHICH ARE AGGLUTINABLE BUT SENSITIVE TO THE "BACTERICIDAL" AGENT

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ALTHOUGH considerable work has been done on the etiology of rheumatoid arthritis, it is still an unsettled problem. Certain workers have reported the isolation of "specific" strains of streptococci from the blood of patients with rheumatoid arthritis but others have been unable to confirm these claims. Our own series of 100 blood cultures failed to reveal bacteria except those of the type reported by Callow.<sup>1</sup> In spite of these conflicting reports it is generally believed that some type of streptococcus plays an important etiologic rôle. In view of the unsatisfactory results usually obtained, other methods were sought for the isolation and differentiation of streptococci obtained from patients suspected of harboring strains of etiologic importance.

In the present paper in addition to discussing isolation methods, we shall compare the ability to produce arthritis in rabbits of strains which either (1) were agglutinated by the patient's blood serum or (2) were resistant to the "bactericidal" action of fresh diluted, defibrinated guinea pig blood or (3) reacted positively or negatively to both tests. It seemed advisable to include a technique for (1) the selective isolation of strains which are more likely to be of etiologic importance and (2) the intensification of differences in the appearances of colonies (because of the possibility that these differences might be associated with important pathologic properties).

Because of the difficulties of preparing stable emulsions of rough strains of streptococci are less suitable for quantitative study. Therefore, they have not been considered in the present report. This becomes important when it is considered that although the pathologic significance of the difference be-

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tween rough and smooth strains is not clearly understood, it is generally considered that smooth strains are more pathogenic for laboratory animals than rough ones. Cowan,<sup>2, 3, 4</sup> Tunnick,<sup>5</sup> and Delves<sup>6</sup> demonstrated that the rough strains which they studied were less "virulent" for mice and rabbits than smooth strains.

The original method used for the selective isolation of smooth strains consisted of plating swabs on blood agar, both aerobically and anaerobically, and isolating and purifying the various types of colonies. Of 1,164 strains of streptococci secured in this manner, 470 (40.4 per cent) were rough and many swabs yielded no smooth strains whatsoever. In certain cases it was necessary to make several consecutive daily examinations before a suitable strain was obtained. Nickel's<sup>7</sup> observation that 59 per cent of the cultures from prostatic secretion did not grow aerobically in primary culture, such as an ordinary streaked blood agar plate or a tube of nutrient broth, prompted a search for better methods of isolation.

It was postulated that, since rough strains tended to form a sediment in broth or other fluid medium, and since streptococci were found to grow better in Rosenow's brain heart infusion, a predominance of smooth strains should be obtainable from the upper layers of brain heart infusion cultures after incubation of the swabs directly in the medium. A group of 284 strains was isolated from blood agar transplants from these upper layers, and only 54 (19 per cent) proved to be rough. Many of the rough strains probably originated from floccules of culture which had been dislodged from the swabs. To eliminate them, the swabs were withdrawn from the tubes after overnight growth and the cultures shaken and allowed to stand in the incubator for an hour or so. This permitted settling of the rough strains. Transplants were made from the supernatant suspensions and the proportion of rough strains was found to be still further reduced. Of 3,500 strains isolated in this manner, only 375 (10.7 per cent) were rough. In a second series, comprising 6,028 strains, 453 (7.4 per cent) were rough. A still more recent series of 2,839 strains contained 131 (4.5 per cent) rough strains. If smooth strains of streptococci were not recovered from the culture, further swabs were examined.

Several points in technic require amplification. Under ordinary working conditions in the average clinical laboratory, a large proportion of streptococcal cultures are impure. In an effort to minimize this defect, the various steps usually employed were investigated, and it was found that several of them invited contamination. Much of it, attributed to air-borne bacteria, was eliminated by the installation of an air filter. However, contaminations continued to occur. It was recalled that, in order to inoculate a Petri dish of culture medium with a platinum loop, it was necessary to make a large number of strokes to cover the plate. This made it difficult to avoid contamination. To overcome this a "spreader" was used. It consisted of an 8-inch glass rod with the rounded end bent at a right angle 4 cm. from the end. These spreaders were sterilized in long manila envelopes with open ends, sealed by gummed flaps. When ready for use the handle end of the envelope was torn off and the spreader lifted out, taking care to avoid contamination of the

bent end from contact with the exposed edges of the envelopes. Three or four strokes of the spreader were sufficient to cover the whole plate.

The second source of contamination was found in the method used to inoculate the liquid medium. A platinum needle was not found satisfactory for harvesting the growths on blood agar because it left the culture piled into a series of ridges. On account of the large number of strokes necessary, it was difficult to avoid contamination. A scraper was devised for obtaining the maximum amount of inoculum from the plates with a minimum number of strokes. This scraper consisted of an aluminum rod used for a handle, and an aluminum blade which was just wide enough to pass through the neck of the bottle containing liquid medium.

With these two instruments and by ventilating the laboratory with filtered air, contamination was reduced to less than 2 per cent of the strains. Growths in the liquid medium were always tested for purity and bile solubility.

When the blood agar plates were incubated in an anaerobic jar, it was noted that the anaerobic environment had intensified the green color produced by certain strains and had favored the development of green pigment by certain strains which did not reveal any when grown aerobically. Furthermore, certain strains which produced hemolytic zones aerobically failed to do so anaerobically but developed green zones instead. It was concluded that the anaerobic method of cultivation permitted a better differentiation of streptococcal colonies than was possible by the aerobic method. It was further noted that, when an aerobic culture appeared to be a pure growth, anaerobic cultivation often revealed several different types of colonies. On certain blood agar plates no colonies were detected by casual examination, but growths were discovered on careful study of the surface of the medium.

The technique, as finally adopted, was as follows. The swabs were placed in tall tubes of brain heart infusion and incubated overnight. The following morning the swabs were withdrawn and the tubes gently rotated. They were then allowed to stand in the incubator for one hour. A loopful of the supernatant growth was transplanted to blood agar, spread by means of the glass spreader, and incubated overnight in an anaerobic jar. The following morning the growths were critically examined by transmitted and reflected light. Representative types of colonies were selected, transplanted to other blood agar plates, and incubated overnight in an anaerobic jar. Impure cultures were further purified\*. The pure substrains were harvested by means of the metal scrapers and transferred to brain heart infusion. Growths usually occurred in less than twenty-four hours but, if the growth in any bottle was poor, it was returned to the incubator.

Cecil et al.<sup>8</sup> reported that the serum of patients with rheumatoid arthritis agglutinated certain "typical" strains of streptococci which they had obtained by blood culture. Believing that the agglutination reaction might be

\*There is a distinct tendency for certain strains to degenerate to the rough type. The intermediate type (SR) gives rise to both rough and smooth daughter colonies but quite often the tendency to degenerate is so pronounced that it is impossible to separate the daughter races without finally losing the smooth forms. For this reason an effort is made to obtain pure cultures as rapidly as possible and to ignore any minimal dissociation rather than attempt to purify the strain at the possible expense of the loss of some of its pathogenic properties.

of differential value, it was applied to all smooth strains of streptococci isolated from suspected foci of infection. The following method was used for the agglutination tests:

Pure substrains of streptococci in Rosenow's brain heart infusion are washed twice with 1.0 per cent phenol in normal saline and resuspended in the latter in concentrations of about 50 billions per c.c. The concentrated suspensions are diluted with normal saline, allowed to stand for one hour to permit settling of the coarser particles, and decanted. If allowed to stand for a longer period, there is a tendency for the suspensions to give weaker agglutination. Even the concentrated suspensions may lose some of their agglutinability on standing. If the tubes are not decanted, the control tubes will often contain a deposit of bacteria and extraneous matter which is difficult to distinguish from specifically agglutinated particles. With the above precaution, however, the controls show only a minimum of sediment. A special technic is necessary for hemolytic streptococci on account of the auto-agglutinative properties of the group. We have used methods described by Spicer,<sup>9, 10</sup> and Mueller and Klise.<sup>11</sup>

Into a series of test tubes is added 1.0 c.c. of serum dilutions ranging from 1:40 to 1:10,240. A saline control is included. To each tube is added 1.0 c.c. of the suspension to be tested. The tubes are shaken gently for two minutes and placed in a water-bath at 50 to 52° C. for two hours. They are allowed to stand overnight at room temperature and the results read the following day. It was found that some of the tubes gave an increased titer when re-examined an hour or so later. This phenomenon was found to be due to the jarring of the tubes consequent to dropping them back into the racks after making the initial observations. Therefore, the tubes are lifted about 1 cm., and permitted to drop against the metal bottoms of the racks. This jarring is sufficient to cause sedimentation of the unstable particles which can be readily observed if the tubes are allowed to stand for one or two hours. After that time the jarring process is repeated and the tubes examined. The examination of the tubes requires critical illumination. This can be improved by placing a black card behind the sediment. The control must always be critically compared with the tubes containing serum dilutions.

Each strain was tested with the serum of the patient from whom it was isolated. In the early work strains agglutinating to a titer of 1:160 or over were used for the preparation of vaccines for the treatment of patients with rheumatoid arthritis. Inagglutinable strains were discarded. While the response to vaccine treatment was favorable in many cases, certain patients were not benefited in spite of the fact that the strains which had been used in the preparation of the vaccines had been agglutinated to a high titer by the patient's serum. This suggested that the agglutination reaction was not sufficiently reliable as a basis for the selection of strains for the preparation of vaccines, possibly because some etiologic strains were inagglutinable or because the serum of certain patients did not contain agglutinins for streptococci.

The possibility of the occurrence of magglutinable pathogenic strains is not a new conception. It is well known, e.g. that freshly isolated strains of typhoid bacilli are sometimes magglutinable. More convincing proof of the existence of magglutinable pathogenic strains of certain bacteria is found in the work of Gilbert and Dacey<sup>12</sup> who isolated *B. abortus* from the blood clot of a patient whose serum did not contain agglutinins for *B. abortus*. Certain strains of *Strept. viridans* isolated by one of us (G. H. C.) from blood cultures of patients with long standing rheumatoid arthritis were not agglutinated by the patient's serum.

In view of the apparent failure of the agglutination reaction for differential purposes other tests were sought.

Among the serologic tests which have been applied to this type of streptococcus is one which is based on the ability of a strain to grow in or survive exposure to fresh blood. Haie<sup>13</sup> pointed out that the ability to multiply in human blood might well be employed to assess the importance of strains isolated from the nose and throat.

Solis Cohen and Boerner<sup>14</sup> believed that it was necessary to use the patient's own blood but Haie<sup>13</sup> tested organisms against normal blood and the blood of the patient and concluded that in general, there was no difference. Fleming<sup>15</sup> was of the same opinion.

For certain species of bacteria the susceptibility to the "bactericidal" activity of normal blood is related to the smoothness or roughness of the strain. This was demonstrated by a number of investigators. Fothergill and Wright<sup>17</sup> showed that the virulent smooth influenza bacillus was completely resistant to the "bactericidal" action of diluted normal unheated serum while the rough organism was easily killed when subjected to similar action. Similar conclusions were reached by Pittman<sup>16</sup>. Grinnell<sup>18</sup> found that a 1:12 dilution of blood killed from 2 to 1,200 of the smooth strains of *B. typhosus* and 1,200,000 of the rough type. This difference was reemphasized by him in a study of the commercial Rawlins strains<sup>20</sup>. Wright and Ward,<sup>16</sup> Fothergill and Wright,<sup>17</sup> Rake,<sup>21</sup> Haie,<sup>22</sup> Todd,<sup>23</sup> and Downie<sup>24</sup> proved that the ability of a strain to grow in fresh blood was correlated with its "virulence" for laboratory animals.

The use of a counted number of live bacteria is a ponderous task and to eliminate this step, the "bactericidal" action was determined by comparing the density of growths obtained from transplants before and after exposure to the diluted blood. As suggested by Gordon and Wormald,<sup>25</sup> cultures of streptococci were mixed with fresh guinea pig blood which had been diluted 1:10, tested for viability, incubated for six hours and retested for viability. The results were difficult to interpret, and there was no significant difference between the growths obtained before and after incubation. While the six hour growths were often lighter than the initial growths identical results were obtained by substituting heated blood for the fresh blood. This indicated that the poorer growths were not due to some factor peculiar to fresh blood and therefore, not to be "bactericidal" principle.

In order to accentuate the effect of the "bactericidal" action, the technic was modified. Fresh, defibrinated, guinea pig blood was diluted 1:4 with sterile saline. Intensification of the "bactericidal" action was still further accomplished by the use of a longer incubation period. However, this rendered the initial mixture unsuitable for control purposes, because it was necessary for the growth obtained from transplants from the initial mixture to stand a day or so until the growth from the final mixture was obtained for comparison. It was considered that, if the active principle was heat labile, the bactericidal action could be estimated by testing the culture simultaneously with heated blood. When a series of strains was tested with 1:4 dilutions of blood, the maximum difference between the growths from heated and unheated blood was found to occur at the end of forty-eight hours.

The technic finally adopted was as follows: Blood was aseptically obtained by heart puncture of a number of guinea pigs, defibrinated with glass beads and pooled. It was then divided into two portions and one of them was heated to 50° C. for thirty minutes to inactivate complement. Higher temperatures, such as are used for the inactivation of hemolytic complement, tended to hemolyze and clot the whole blood. Portions of the heated and unheated blood were separately diluted with three volumes of sterile saline. Dilution of the defibrinated blood with nutrient media, instead of with saline, confused the readings. Two rows of sterile plugged tubes were placed in a rack. To each tube in the front row was added 0.5 c.c. of the diluted unheated blood. The tubes in the rear row received 0.5 c.c. of diluted heated blood. The latter served as controls. A loopful of each overnight culture in brain heart infusion, obtained as previously described, was added to each of a pair of tubes, consisting of a tube of unheated blood and a heated blood control tube. The tubes were thoroughly shaken and incubated for forty-eight hours. After shaking, a loopful from each tube was streaked on blood agar in such a way that the growths from transplants from each pair of tubes could be compared. After overnight incubation, the growths from the fresh blood were compared with growths from the control tubes.

If the growths from the heated and the unheated blood were similar, the reactions were recorded as ++++; if the growth from the fresh blood was slightly diminished, the reaction was recorded as +++; a poor growth from the fresh blood as contrasted with a good growth from the heated blood was recorded as ++ or +; while the absence of growth from the fresh blood as compared with a good growth from the heated blood indicated a negative reaction.

This test, hereafter referred to as the "bactericidin" reaction, was applied to all smooth strains of streptococci isolated, as previously described, from the suspected foci of infection of patients with rheumatoid arthritis.

It is not intended to consider the term "bactericidin" as implying a conviction that the active principle of fresh blood has a "bactericidal" as compared with a "bacteriostatic" action, but until this question has been settled, customary usage will be followed and the term "bactericidin" used to denote the active principle of fresh blood. Organisms highly resistant to the "bactericidal" action (++++ or +++ reactions) are referred to as "bactericidin

positive," while those which are susceptible to the "bactericidal" action (++ or negative reactions) are referred to as "bactericidal negative" strains. The resistant strains are considered "positive" because they appear to be more significant than strains which are sensitive to bactericidal.

Strains which resisted bactericidal were compared with strains which were agglutinated by the patient's serum by injecting live cultures into rabbits. In certain instances more than one type of strain (in regard to the agglutination and bactericidal tests) was obtained from a single patient. These strains were also compared by animal experimentation. The strains selected for study were either (1) magglutinable but resistant to bactericidal, (2) agglutinable but sensitive to bactericidal, (3) reacting positively to both tests, or (4) reacting negatively to both tests.

They were grouped as follows:

GROUP	REACTION		NO. OF STRAINS TESTED
	ACID	BACT	
1	0	+	19
2	+	0	10
3	+	+	7
4	0	0	9

Each strain was grown in brain heart infusion overnight. The following day 0.5 to 1.0 cc was injected intravenously into a rabbit. Injections were repeated about every four days and were increased as tolerated. In those instances where the rabbits showed marked symptoms after the injection (such as loss of weight, loss of appetite, listlessness, stiffness, etc.) the interval was lengthened and the dose reduced until these symptoms were reduced to a minimum. The procedure is somewhat different from that employed by Hadjopoulos and Burbank<sup>26</sup> who used doses of 1.0 cc of a twenty-four hour broth culture daily for four consecutive days.

Injections were continued until the rabbits showed evidence of chronic arthritis. In some cases this required only five injections; in others as many as 20 or more were needed. Joint symptoms usually appeared after the fifth or sixth injection, but in one instance they were not noted until after the seventeenth injection. The rabbits frequently developed further listlessness, anorexia, and loss of weight. There was usually a period of acute arthritis of varying duration developing from about seven to ten days after the initial injection, following which the rabbit appeared normal. The development of acute arthritis was not considered of significance. Rabbits were classed as having arthritis when the disease progressed to the chronic stage with deformities and permanent joint disease confirmed by roentgen ray examination.

Roentgen ray studies were made in over 80 per cent of the rabbits. In some instances they showed only a haziness of the joint with swelling of the soft tissues, indicating an early lesion. In others there was absorption of the cartilage with proliferation of the bone, which varied in degree in different rabbits. A diagnosis of arthritis was made only when these reactions were pronounced. In some instances, there was complete disintegration of the



joint. Bowing of the long bones was frequently observed, but this was not considered of significance as it is a normal occurrence in certain rabbits.

Autopsies were made of certain animals in each group and histologic examination of discs of ground bone was made of representative joints from rabbits of each group. In general, all the bone discs showed varying degrees of decalcified bone.

Bone discs were also made from different sections of all the larger bones from certain rabbits with arthritis. All the discs showed evidence of varying degrees of repair of preexisting lesions. These changes were interpreted as evidence that the bones had been affected, not only at the joints, but also throughout their entire length. Sections of the synovial membranes revealed chronic synovitis.

Agglutination reactions with the offending strain were made at irregular intervals in forty-six of the rabbits which developed arthritis. The reactions were positive, i.e., the titer was 1:160 or over, in thirty-eight rabbits (82.6 per cent).

The following summaries illustrate the effects produced by the different types of streptococci:

*Group I Strains.*—In this group there were nineteen strains which were not agglutinated by the blood serum of the patient from whom they were obtained but were resistant to bactericidin. They were injected into rabbits in graded amounts as previously described. Three strains were injected into each of two rabbits to compare their ability to produce arthritis in different rabbits. The rabbits were more severely ill, and more frequently developed diarrhea, lost weight and died from the effects of smaller doses of culture than those rabbits which were injected with strains of the other groups. Four rabbits died during the course of the injections and are not included in the tabulations. All the remaining rabbits developed arthritis and, in almost every instance, it appeared more quickly and was induced by smaller doses than were required for the production of chronic arthritis by other groups of strains. The strains of this group produced arthritis in from 4 to 17 injections, the maximum amount being from 0.5 to 12.0 c.c. Arthritis developed in from seven to seventy-four days after the initial injections.

*Group II Strains.*—This group consisted of 10 strains which were agglutinated by the serum of the patient from whom they were obtained but were sensitive to bactericidin. Graded amounts of cultures were injected into ten rabbits as previously described. Two of the rabbits died during the course of the injections and are not included in the tables. Arthritis developed in seven rabbits (87.5 per cent). After its development they were kept for a prolonged period and several of them died, presumably from arthritis because they showed deformities, marked loss of weight and loss of appetite. Death was apparently due to exhaustion.

In two instances, the strain was injected into each of two rabbits for comparative purposes. Essentially similar characteristics were noted in both rabbits of each pair.

The rabbits inoculated with this group of strains required from nine to

sixteen injections, the maximum amounts ranging from 10 to 200 cc. Arthritis developed in from nine to one hundred and eleven days.

*Group III Strains*—The seven strains of this group were agglutinated by the blood serum of the patients from whom they were obtained and resisted bactericidin. Most of the strains were injected into more than one rabbit for comparative purposes. One of them died during the course of the injections. Arthritis developed in eleven rabbits (91.66 per cent). The morbidity, maximum dose required (0.5 to 100 cc) and total amount used (50 to 104 cc) for the production of chronic arthritis with this group of strains did not differ appreciably from those used of magglutinable strains which resisted bactericidin. However, symptoms of arthritis did not appear until from thirteen to eighty days, which was somewhat longer than was required for the magglutinable strains which resisted bactericidin. From four to sixteen injections were required for the production of arthritis in rabbits with this

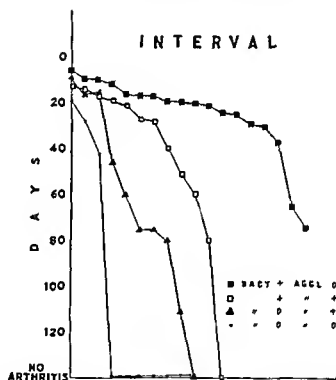


Chart 1—Comparison of intervals between initial injection and the appearance of chronic arthritis in rabbits injected intravenously with strains of *Streptococcus viridans* which differ in their reactions to the agglutination and bactericidin tests.

group of strains. When strains were injected into more than one rabbit, no essential difference was noted except that arthritis was not produced in one rabbit by a strain which had produced arthritis in two other rabbits.

*Group IV Strains*—This group consisted of nine strains which were not agglutinated by the serum of the patients from whom they were isolated and which were sensitive to bactericidin. The cultures were injected intravenously into rabbits as previously described. Arthritis developed in only three (30 per cent). The interval required for its development (nineteen to forty-two days), the number of injections required (11 to 15), the total amount used (85 to 105 cc), and the maximum dose (10 to 12 cc) were more than was necessary for the production of chronic arthritis with the other groups of streptococci. When one strain was injected into each of two rabbits, there was no essential difference in its arthritis-producing ability.

Charts 1, 2 and 3 illustrate the differences in the characteristics of the arthritis-producing ability of the four groups of strains. Chart 1 illustrates the interval between the first injection and the development of chronic arthritis. Each rabbit injected with Group I strains developed chronic arthritis in a shorter period of time than rabbits injected with strains of the other groups. Group III strains required longer periods of time than Group I strains which differed from them only by their agglutinability. Strains which were sensitive to bactericidin required still longer periods, particularly if the agglutination reaction was negative.

When strains which resist bactericidin are compared on the basis of their agglutination reactions, it would be expected that agglutinable strains would produce arthritis in rabbits in a shorter period of time than inagglutinable

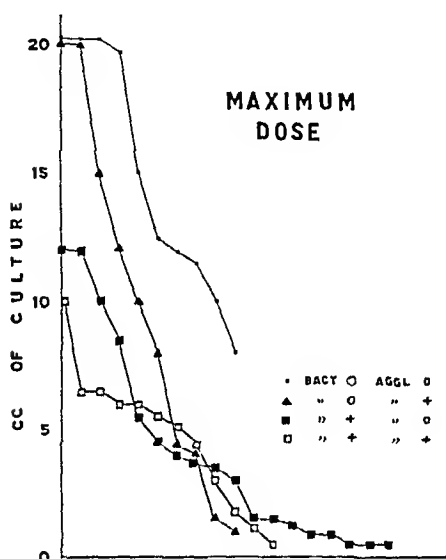


Chart 2.

Chart 2.—Comparison of the maximum doses used for the production of chronic arthritis by the intravenous injection of strains of *Streptococcus viridans* which differ in their reactions to the agglutination and "bactericidin" tests.

Chart 3.—Comparison of total volumes of culture required for the production of chronic arthritis in rabbits by strains of *Streptococcus viridans* which differ in their reactions to the agglutination and "bactericidin" tests.

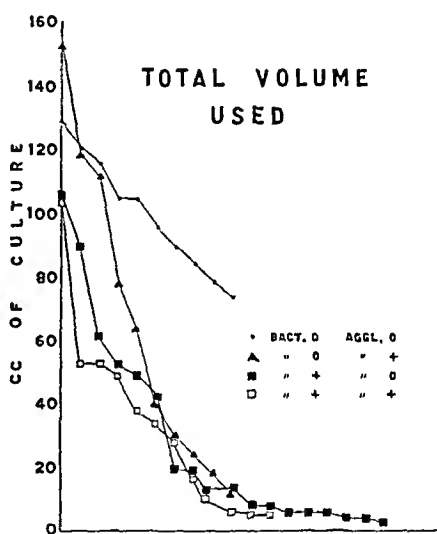


Chart 3.

strains. However, in the series of cultures tested, the inagglutinable strains which resisted bactericidin produced chronic arthritis in a much shorter time than the agglutinable strains which resisted bactericidin.

The maximum dose was fairly characteristic for each of the four groups of strains and was distributed in the following order:

AMOUNT REQUIRED	BACTERICIDIN REACTION	AGGLUTINATION REACTION
Highest maximum dose	0	0
Second largest maximum dose	0	+
Third largest maximum dose	+	+
Least maximum dose	+	0

The total volumes of culture required for the production of arthritis in rabbits with the different groups did not show a marked difference (Chart 3), but the same sequence was noted as with the maximum dose. A considerably larger amount of culture was used when the strains reacted negatively to both tests. In this case the larger total volume of culture required was characteristic and was considerably larger than that used for the other groups of strains.

The difference in the rate and severity of infection induced by those strains which resisted bacterioidin as compared with those which were sensitive to it is well illustrated by a comparison of the following typical experiments (Tables I and II).

TABLE I

PROTOCOL OF RABBIT INJECTED INTRAVENOUSLY WITH *Streptococcus viridans* STRAIN 341  
(BACTERIOIDIN REACTION ++++ AGGLUTININ TITER LESS THAN 1:160)

DATE	AMOUNT INJECTED	OBSERVATIONS
April 17	0.5 cc	
18	1.0	
19	1.5	Diarrhea Stiffness in both hind legs, mostly in hips
20	2.0	
21	2.0	Definite stiffness in right hip and knee Listless appetite poor
24	3.0	Stiffness slowly decreased from this time
28	4.0	
May 2	5.0	Stiffness disappeared
4	6.5	
11	7.5	
18	8.5*	Left knee swollen and tender Decided limp
Injections discontinued		
May 24	-	Carried left leg when moving Some stiffness in spine
June -	-	No change in leg Stiffness of spine gradually increased
June 28	-	Marked deformity of left rear leg Marked stiffness of spine with head fixed to one side Chloroformed Roentgeno- grams made Autopsy

\*Chronic arthritis considered as beginning at this time

Strain 341 was resistant to bacterioidin. It was injected into a rabbit as previously described. Diarrhea developed after the third injection (1.5 cc), and acute arthritis was noted after the fifth injection (2.0 cc). After the eleventh injection (8.5 cc) which was given one month after the first the left knee was swollen and tender, and there was a decided limp. The injections were discontinued but arthritis persisted until the rabbit was killed forty one days later.

Strain 8012 was sensitive to bacterioidin but was agglutinated to a titer of 1:20480 by the blood serum of the patient from whom it was isolated. It was inoculated into a rabbit as previously described. The initial dose was 0.5 cc. This was rapidly increased until the seventh dose (6.5 cc) was reached in twenty one days. The rabbit was listless and there was some generalized stiffness which disappeared thirteen days later. When the dose of 15.0 cc was reached (nineteen days after the disappearance of the acute symptoms), there was a slight stiffness and slight tenderness in the right knee. However definite stiffness was not noticeable until after the fifteenth injection (20.0 cc) which was given twenty three days later.

The results are summarized in Table III.

In an attempt to compare the effect of different types of strains from each of several patients, 23 strains representing 10 groups of 2 or 3 strains were injected into rabbits. Each group was isolated from a single patient. As

TABLE II

PROTOCOL OF RABBIT INJECTED INTRAVENOUSLY WITH *Streptococcus Viridans* STRAIN 8012  
(BACTERICIDIN REACTION NEGATIVE, AGGLUTININ TITER 1:20,480)

DATE	AMOUNT INJECTED	OBSERVATIONS
December 12	0.5 c.c.	
13	1.0	
14	1.5	
15	3.0	
19	4.0	
26	5.0	
January 3	6.5	Listless. Some generalized stiffness.
10	7.0	
16	8.0	Stiffness has disappeared.
23	10.0	
30	12.0	
February 4	15.0	Slight stiffness in right knee. Slight tenderness.
14	18.0	
20	19.0	
27	20.0*	Definite stiffness in right knee and right hip.
March 5	20.0	
Injections discontinued		
March 26	-	Carried right leg when moving and favored left fore paw. Stiffness gradually increased in right rear leg and left fore paw. The left rear leg also became involved. Extremely crippled and moved with difficulty.
July 10	-	Chloroformed. Roentgenograms made. Autopsy.

\*Chronic arthritis considered as beginning at this time.

TABLE III

GROUP	BACTE- RICIDIN	AGGLU- TININ	MEAN INTERVAL (IN DAYS) *	MEAN MAXIMUM DOSE (IN C.C.)	MEAN TOTAL VOLUME USED (IN C.C.)	MEAN NO. OF INJEC- TIONS
1	+	0	25.0	4.1	28.2	9.9
2	0	+	54.0	9.6	60.8	13.1
3	+	+	33.7	4.7	34.0	11.9
4	0	0	30.0†	14.9	99.8	13.7

\*Before the development of arthritis.

†On the basis of only three strains.

previously stated, the object of studying this group was to determine if strains isolated from the same patient but differing in their reactions to the two in vitro tests differed in their ability to produce arthritis in rabbits. Three rabbits dying after the third or fourth injection and one rabbit dying following an injury are not included in the table (Table IV).

The production of arthritis in rabbits by the injection of strains of these groups was apparently correlated with the serologic type of the strain. In general, if the strain resisted bactericidin, arthritis was produced in a shorter

time, a smaller number of injections were required, and a smaller maximum dose was necessary than was the case with strains which were sensitive to bactericidin

TABLE IV

COMPARISON OF THE EFFECTS OF THE INTRAVENOUS INJECTION OF RABBITS WITH DIFFERENTLY REACTING STRAINS OF *STREPTOCOCCUS VIRIDANS* FROM THE SAME PATIENT

PATIENT	STRAIN NUMBER	BACTERICIDIN REACTION	AGGLUTININ TITER	INTERVAL (DAYS)*	MAXIMUM DOSE (cc)	TOTAL AMOUNT (cc)†
F M	8963	++	1 10,240	50	6.0	52.5
	8965	++++	1 640	28	1.8	4.5
M J	343	0	1 640	13	4.5	29.3
	341	++++	0	37	8.5	49.5
	339	++++	0	15	3.5	11.0
R T	719	++++	1 640	10	0.5	5.0
	712	+++	0	14	1.5	12.2
A S	9972	++++	0	17	0.5	6.5
	9973	0	1 640	7	8.0	55.6
C K	8177	+++	1 320	30	5.5	16.7
	7842	++++	0	24	4.5	19.2
L D	708	0	1 640	15	1.5	16.0
	744	+++	0	10	0.5	3.5
W H	502	++++	0	30	4.0	53.6
	543	++++	1 640	10	5.0	39.3
T H	703	++++	0	17	0.5	2.5
	704	++++	1 640	5	1.5	7.9
	750	0	1 1,280	6	1.5	13.7
E K	528	++++	0	2	3.0	13.4
	521	+++	1 160	7	4.0	19.5
	523	0	1 640	3	1.0	10.0
M L	9536	++++	0	76	12.0	10.5
	9523	++++	1 1,280	20	12.0	87.1

\*Interval before the appearance of chronic joint symptoms

†Total amount of culture used

## SUMMARY

Methods are described for the selective isolation of smooth strains of streptococci from suspected foci of infection of patients with chronic rheumatoid arthritis. When isolated, these strains were tested for agglutinability by the serum of the patients from whom they were isolated. Because vaccines prepared from certain strains with high agglutinin titers produced no therapeutic response when injected into patients with chronic rheumatoid arthritis it was suggested that these agglutinable strains did not possess immunogenic value or that strains of etiologic significance may not have been agglutinable by the patient's serum.

From this it was reasoned that other factors might be involved in the antigenic structure of streptococci isolated from patients with rheumatoid arthritis. It seemed possible that some magglutinable strains might contain

important factors which were not present in the agglutinable strains under consideration. Attempts were made to differentiate streptococci by other *in vitro* tests and to compare the reactions to these tests with the ability of the strain to produce chronic rheumatoid arthritis in rabbits.

One of the reactions studied was based on the "bactericidal" action of normal blood and was applied to a number of strains of streptococci. Strains were differentiated on the basis of their reactions to this "bactericidin" test and their agglutinability, in titers of 1:160 or over, with the serum of the patient from whom they were obtained. Those strains which reacted to only one of these tests were selected for animal inoculation. The respective abilities of these strains to produce arthritis in rabbits were determined by the intravenous injection of live cultures in doses which were progressively increased until chronic arthritis developed or until the volume injected each time reached 15 to 20 c.c. Strains reacting positively or negatively to both tests were used for comparison with strains reacting positively to only one test. In addition, ten groups of strains, each containing different types of strains isolated from a single patient, were similarly injected into rabbits to determine if there were differences in the arthritis-producing ability of the various strains which are closely associated in a suspected focus of infection. Certain strains were injected into more than one rabbit for comparative purposes.

Chronic arthritis was produced in 90 to 100 per cent of rabbits by strains which either resisted the "bactericidal" action of fresh, diluted, defibrinated guinea pig blood or were agglutinable by the patient's serum. However, it was produced in only 30 per cent of the rabbits by strains which did not resist the "bactericidal" action of fresh, diluted, defibrinated guinea pig blood and which were inagglutinable by the serum of the patient from whom they were obtained.

In those instances where several strains differing in their reactions to these two tests were isolated from the same patient and injected into rabbits, arthritis was produced in rabbits by the strains which were either agglutinable, in a dilution of 1:160 or over of the patient's serum, or which resisted the "bactericidal" action of fresh, diluted, defibrinated guinea pig blood; but the rate of development differed considerably and was correlated with the reaction of the strain to the agglutination and "bactericidin" tests.

When all the different groups are considered, strains which reacted only to the bactericidin test produced arthritis in rabbits in a shorter period of time and required fewer injections, a smaller maximum dose, and a smaller total volume of culture than strains of any other group.

Next in order were those strains which, in addition to their positive reactions to the bactericidin test, were agglutinable by the serum of the patient from whom they were isolated.

When the agglutination reaction alone was positive, the strains required a longer interval, a larger maximum dose, a larger total volume of culture, and more injections than the two previous groups.

Finally, strains which reacted negatively to both the bacterioidin and agglutinin tests took the longest time for the production of arthritis in rabbits, required the largest number of injections, the largest maximum dose and the largest total volume of culture of any group of strains.

While consideration has been given only to the relationship between the agglutination and "bacterioidin" reactions of streptococci and the ability of the strains to produce chronic arthritis in rabbits, it is possible that these factors may also be associated with other pathogenic factors. For example, there was evidence that those strains which resisted the "bacterioidin" action of diluted, fresh defibrinated guinea pig blood were more toxic for rabbits than those strains which were sensitive to the "bacterioidin" action. No such relationship could be demonstrated between the agglutination reaction and toxicity for rabbits.

#### CONCLUSIONS

Strains of streptococci which are resistant to the "bacterioidin" action of fresh, diluted, defibrinated guinea pig blood are capable of producing chronic arthritis in rabbits. Strains which are not resistant to the "bacterioidin" action but which are agglutinable by the serum of the patient from whom they were obtained are also capable of producing chronic arthritis in rabbits, but require larger doses of culture, a longer period of time, and a larger number of injections than those strains which are resistant to the "bacterioidin" action. Strains which do not respond to either the "bacterioidin" or the agglutinin test are often incapable of producing chronic arthritis in rabbits under the conditions used in these experiments.

Therefore, (1) the reaction of a strain to the "bacterioidin" action of fresh, diluted, defibrinated guinea pig blood, and (2) the agglutination reaction with the patient's own serum are of value for the study of streptococci isolated from suspected foci of infection of patients with chronic rheumatoid arthritis.

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# LABORATORY METHODS

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## THE EFFECT OF HYDROGEN ION CONCENTRATION UPON THE DETERMINATION OF CALCIUM IN BLOOD SERUM PHOSPHOMOLYBDIC ACID CENTRIFUGATES\*

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VON BERENESY<sup>1</sup> has reported a chemical method for the quantitative determination of nonprotein bound calcium in blood serum, in which the protein is precipitated with phosphomolybdic acid and the filtrate analyzed for calcium. He found 60 to 65 per cent of the calcium in the filtrate, and he assumed that the remainder, which was precipitated along with the protein, was actually bound to the latter. In order to obtain sufficient material from small amounts of rabbit's blood Hermann<sup>2</sup> modified the method by centrifuging, instead of filtering off the precipitate resulting from the addition of 4 c.c. of 2.5 per cent phosphomolybdic acid to 2 c.c. of serum.

In order to test the assumption of von Berenesy and Hermann that the calcium precipitated with the protein was actually bound to it, Rosenthal<sup>3</sup> added increments of a calcium chloride solution to a phosphomolybdic acid serum mixture, and was unable to recover the added calcium quantitatively in either centrifugate or filtrate. He therefore concluded that protein precipitation by phosphomolybdic acid, followed by calcium determinations in the filtrate or centrifugate and total calcium determinations on the serum, gives no information as to what part of the serum calcium is in the bound or the unbound form.

In the course of a pharmacologic investigation on the blood serum calcium of cats and dogs, the von Berenesy-Hermann method was given a trial for the determination of nonprotein bound calcium. It was observed, however, that the appearance of the centrifugates obtained from the addition of two parts of 2.5 per cent phosphomolybdic acid to one part of serum varied with individual animals of the same species, in that some were clear, while others were opalescent or cloudy, indicating incomplete precipitation of protein, which was demonstrated to be true upon the addition of more acid to the suspected centrifugates. Calcium determinations on the oxalate precipitates of these centrifugates yielded very confusing results, and it was decided, therefore, to investigate the relationship of hydrogen ion concentration to calcium content in serum phosphomolybdic acid centrifugates.

Various studies were conducted on blood serum. In this paper the results obtained with one cat and two dogs are reported. The cat was anesthetized with

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sodium barbital and the dogs with nembutal. The animals were bled by cannula from the carotid artery. Serum and phosphomolybdic acid were mixed in various proportions, allowed to stand about half an hour, and thoroughly centrifuged. The hydrogen ion concentration of the decanted centrifugates was determined with the quinhydrone electrode, and their calcium content, as well as that of the trichloroacetic acid centrifugate for total calcium, was determined by the Clark and Collip<sup>4</sup> modification of the Kramer-Tisdall method.

The results of these studies are shown in Fig. 1. All centrifugates with a hydrogen ion concentration on the alkaline side of approximately pH 4.1 were opalescent or cloudy, insufficient acid being present to cause complete protein precipitation. Some of the suspended protein came down with the oxalate precipitate, so that in titrating, permanganate was reduced by protein as well as

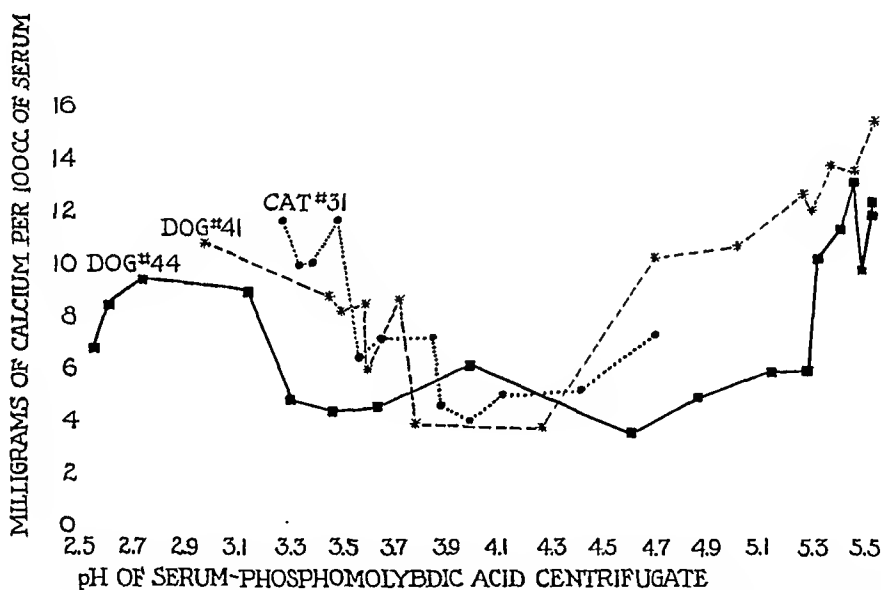


Fig. 1.—The relation of pH to calcium recoveries in serum phosphomolybdic acid centrifugates. (Total Calcium Values: Cat 31, 11.9; Dog 41, 12.2; and Dog 44, 12.1 mg. per 100 c.c. of serum.)

by the oxalates of protein bound and nonprotein bound calcium. As a result, calcium calculations based on the amount of permanganate reduced were too high, the values in some cases being greater than the amount obtained in the separate analysis for total calcium. On the other hand, all centrifugates with a hydrogen ion concentration on the acid side of pH 4.1 were clear, indicating that there was also an irregular but progressive increase in calcium, due to removal of calcium from protein by excess acidity. Therefore, in those cases where the acidity exceeded the minimum necessary for complete protein precipitation, permanganate titration of the oxalate precipitate measured not only the original nonprotein bound or free calcium, but in addition calcium which had been released from the protein precipitate by the excess acid.

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## SUMMARY AND CONCLUSIONS

The addition of two parts of 2.5 per cent phosphomolybdic acid to one part of serum, the procedure used in Hermann's modification of von Berenesy's method for determining unbound calcium, does not always produce the exact hydrogen ion concentration needed for complete protein precipitation. When protein precipitation is incomplete, permanganate titration for calcium yields high values. High values are also obtained for unbound calcium when the acidity is greater than the minimum needed for complete protein precipitation, since this causes the removal of calcium from the precipitate. Since the amount of protein in blood serum is known to vary widely under different conditions,<sup>6</sup> it is obviously impossible to produce a hydrogen ion concentration from the addition of a fixed amount of phosphomolybdic acid that will quantitatively precipitate the serum proteins and at the same time not remove bound calcium from the precipitate. It therefore appears that the von Berenesy-Hermann method, employing a fixed amount of acid, is not satisfactory for the determination of nonprotein bound or free calcium in blood serum.

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## THE TECHNIC OF MOLDING AND CASTING FOR THE MEDICAL SCIENCES\*

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**D**EFINITION.—Molding and casting are processes of reproducing an object in form, texture, and sometimes color identical in appearance to the original from which the copy or reproduction is made. To obtain such casts there are two main processes to be undertaken. The first is that of producing the mold, or negative. The second is the making of the cast or positive from the mold. In other words: a mold is an impression of an object such as a face, a hand, a coin; while a cast is an impression of the mold that was made of the object such as a face, a hand, or a coin. In science, the finished cast or positive is sometimes called “moulage,” a word of French origin (*mouler*) meaning to cast, or to mold. The words “negative” and “positive” will also be used in this text as a means of simplification. I prefer the word “moulage” to express both molding and casting as a unit, though the word “casting” is often used alone to express both processes.

**Purpose.**—The object of this text is not to dilate upon the history or application of molding and casting or the moulage process, but to describe practical methods of compounding correct formulas. One might say, however, the growing belief in visual education is a sufficient reason for its intelligent and successful application. Every physician realizes the value of the “before and after” representation of a surgical or medical case. The three dimensional model, naturally colored, leaves little to the imagination and gives practical evidence not apparent in photographs and drawings. The physician and dentist find them well worth while where plastic and oral surgery is done to change the facial contours. They are invaluable in lecture and reference work. They are a permanent and natural means of preserving a case record. By means of photography and photomechanical reproduction for publication, pictures in black and white, or natural colors, of such models can be obtained on paper, loose, or in book form for more convenient preservation and dissemination.

**Compositions for Making the Negative.**—The two main substances used for obtaining the negatives of medical subjects are *plaster of Paris* (gypsum), and a hydrocolloidal composition which we will call “*agar mold*,” and “*agar composition*” because its important constituent is agar. Sometimes the *wax mold* is employed. For inanimate and nonprotoplasmic objects, the *glue mold* is often used; and sulphur molds are rarely used. Other substances such as metal, rubber, baked sand, terra cotta, and similar materials are employed for mold making in commercial manufacturing. In medicine there are cases

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in which plaster should be used alone and other cases in which it is more convenient to use agar composition, as this can be easily removed from undercuts. It is not advisable to try to use one substance for all cases. Sometimes plaster is used as a shell to support an agar mold. Plaster of Paris in dry powdered form becomes rigid or *set* when mixed with water. This setting takes place in a relatively short time. If properly used, it will give a negative that has an infinite amount of desirable detail from which the positive is finally made. However, its great disadvantage is the fact that it is not plastic or pliable as is the agar or glue mold.

*Molds in General*—One of the best descriptions that has been written on the making of facial masks and molds as well as casts of the inside of the

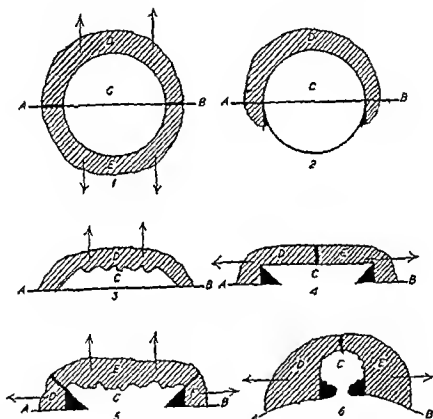


FIG 1—Diagrams of molds. The white area represents the rigid subject that is being cast, the shaded area represents the molds and the black areas the undercuts. Arrows point in the directions that the molds or their pieces are moved to separate them from the original subject or cast.

mouth appears in *Practical Orthodontia*<sup>1</sup> by Dewey. This author considers no casting materials other than those used in plaster and glue molds with which most casters are familiar.

Many books on modeling and sculpture, such as the ones by Toft<sup>2</sup> and Glass<sup>3</sup> give interesting points on the technique of producing plaster and glue molds. Much information in the nature of formulas may be found in *Dental Formulary*<sup>4</sup>. A most informative book, from a technical standpoint, is by Frederick<sup>5</sup> who writes about the making of plaster molds of many kinds. He also gives some description of wax and sulphur molds. Much has been written about plaster molds in hundreds of books. It is for this reason that little will be said about them in this article.

*Undercutting*—The main difficulty which the caster experiences is that

caused by *undercutting*. An *undercut* is the part of the original subject or mold that is to be cast which prevents the impression from slipping freely away or separating.

For example, let us consider Fig. 1. The white areas, or *C* in these diagrams, represent the subject to be cast. The shaded areas represent the mold and the black areas represent the undercuts. The undercuts or black areas are always a part of the mold and must be considered as such. The arrows denote the directions in which the mold must be pulled to separate it from the subject.

Let us consider these diagrams separately. *C* of Part 1 can represent a sphere of wood or stone. If we wish to cast such a subject, it should be done in two pieces. Such a mold is called a piece mold. The line *AB* is imaginary and passes directly through the center of the sphere, which would divide the mold into two separate pieces, *D* and *E*. Should one-half of this piece mold lap over beyond the imaginary line *AB*, Part 2, then it would be impossible to separate the sphere from a rigid mold without breaking the mold. The black area in Part 2 represents the undercut that would prevent the separation. *C* of Part 3 represents the type of subject that has no undercuts. Therefore, for such an object, it is necessary to make a mold of only one piece. The line *AB* now becomes a flat surface such as a table top upon which the subject rests. In Part 4, consider that the subject is attached to the surface *AB*. However, *C* has a smooth top surface. In such a case a mold of two pieces can be made of a subject providing it is possible to separate the two pieces of mold by pulling them in the opposite directions as indicated by the arrows. It is only necessary to separate them far enough to be relieved of the undercut. In Part 5, we have a subject similar to that in Part 4. However, the top surface is irregular rather than smooth. In such a case it becomes necessary to make a mold of three pieces as the rough surface would prevent drawing a two piece mold to the right and left.

In Part 6 of Fig. 1, let us consider *C* to be a pedunculated tumor attached to the surface of the body *AB*. Such an object can be made in a two piece mold *D* and *E*.

*Sulphur Molds*.—Sulphur molds are unsuitable for use in making impressions of living or dead tissue and are therefore not considered in detail. They are sometimes used for reproducing impressions of coins, medals and medallions, or other delicate reliefs in which there is no undercutting. A wall of thin metal is placed around the subject to be cast and both the subject and wall are shellacked or greased. Eight parts of sulphur and one part of iron filings are melted together and allowed to cool. This mixture while still in a liquid state is poured over the subject, and blown into depressions. When completely cooled, the mold is greased and a plaster impression is made from the sulphur mold.

*Wax Molds*.—Frederick in his book, which was first published in 1899, writes of wax molds as follows: "Molds may be successfully made of wax, usually of small or delicate objects, as fruit, flowers, small animals, coins, medals, or of the hands and face. Take one part of rosin and three parts of

white wax. Melt them in a hot water bath, as a glass jar placed in boiling water, and heat to the boiling point. Or melt paraffin wax (as a paraffin candle), and add a little sweet oil and whiting."

He advises that the face should be greased as he has previously described (for making plaster molds) and the wax can be applied as soon as it cools to a bearable point. He continues "Brush it on quickly to a thickness of about  $\frac{1}{8}$  inch, and, as it almost immediately hardens, an outer shell or case of plaster can be cast before the model feels compelled to move. Remove the case from the wax and the wax from the model, replacing it in the case at once to prevent warping or breaking, and make the cast as soon as possible."

In the June 1926, issue of *The Journal of the American Dental Association*, Golden<sup>6</sup> describes his method of making wax molds of the face. In a later issue<sup>7</sup> of the same publication, Dr. Golden condenses his first article and discusses other materials in use for the same purpose, such as plaster and "Negocoll." In short his wax mold method is as follows. A mixture of waxes is prepared which he uses to spray on the patient's face to obtain the mold. This mixture which he terms B 11 contains

Paraffin wax	55% (Melt point 51.7° C)
Bayberry wax	20% (Melt point 40.9° C)
Carnauba wax	5% (Melt point 84.8° C)
Stearic acid	20% (Melt point 69.9° C)
Resulting melting point of mixture 52.97° C	

According to the author, no preliminary preparation of the skin surfaces is necessary. However, vaseline can be used on the eyelashes and brows to prevent the hairs from sticking to the mold.

For spraying the wax mixture on the face, the author used a DeVilbiss type CB spray gun. It seems that any spray gun, such as is used for spraying oil paint, should be suitable for this purpose. The first layer of wax was applied with an air pressure of only four to six pounds. When the air pressure is increased above this point the deposit becomes uneven and a mist results. This first layer serves as an insulator for a second layer which can be about half an inch in thickness. The latter can be applied with a spatula when the wax is in a congealing state. Little rubber bags filled with ice or dry ice (magic ice) serve well for cooling or chilling the wax mold at different stages of the process. About one inch of wax should be applied, after which the mold is supported with a plaster of Paris shell, or mother mold, as seen in Fig. 2.

This same author gives the following wax composition which can be painted on the face with a camel's hair brush rather than sprayed.

Bayberry wax	50% (Melt point 40.9° C)
Paraffin	25% (Melt point 51.7° C)
Stearic acid	25% (Melt point 69.9° C)
Resulting melting point of mixture—41.7° 42.8° C (Saybolt test)	

Golden continues "As this combination continues plastic until possibly thirty degrees above its melting point it is adaptable for use in brush applications to take impressions and advisable to use for partial facial impressions."



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extent. By their use a great amount of undercutting is taken care of in the stretching or bending of the mold. Glue and gelatin molds have a rubber-like quality of springing back into their original form when once they are removed from the cast. This is accomplished by stretching the negative away from the cast in order to remove it from the mold. In other words, the elastic quality of glue or gelatin allows it to be pulled from beneath projections and around bulging surfaces after which it immediately springs back into its proper position.

Due to the fact that the proper concentration of gelatin or glue and water requires a great amount of heat to bring it to the liquid state for pour-

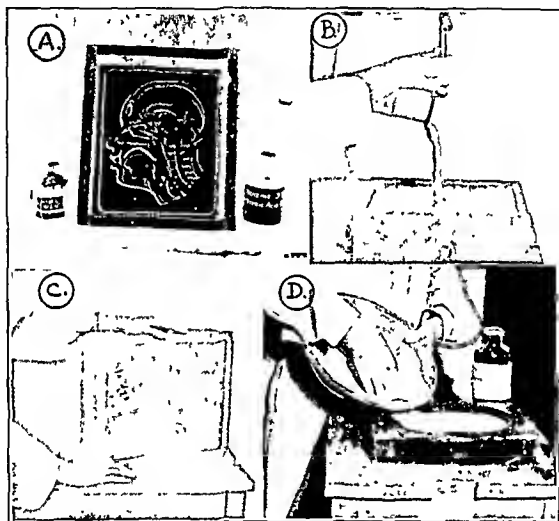


Fig. 3.—The glue mold process. A, The plaster subject that is being cast. This is shellacked then painted with a mixture of stearic acid 1 part and benzine 3 parts. B, Pouring the hot glue over the plaster cast. C, The outside of the mold after the glue has cooled and set. The original cast is removed from the mold. D, The mold is painted with the stearic acid-benzine mixture and poured full of plaster. The duplicate cast is removed immediately upon setting, otherwise the heat created by the setting plaster is likely to melt the glue of the mold.

ing, it is, therefore, unsuitable for making a mold directly over living or dead tissue. Furthermore, it takes a considerable time to cool and set, which makes it more unsuitable. In the case where heat is likely to have a destroying effect, it is necessary to use a substance that can be applied in a liquid state, but which will not burn the subject, yet will set, taking the negative form of the tissue upon which it is applied.

The *agar mold* has the qualities of melting at slightly below 100° C. (212° F.). However, it does not return to its semisolid or plastic state until it cools to a temperature of about 42° to 44° C. (108° to 112° F.). Since this is

just about body temperature ( $37^{\circ}\text{C}$ . or  $98.6^{\circ}\text{F}$ .), agar, which has many of the other qualities of glue, suggested itself for plastic casting of living subjects. This composition can be applied in a liquid state before it cools to its setting point, without doing harm to living or dead tissue.

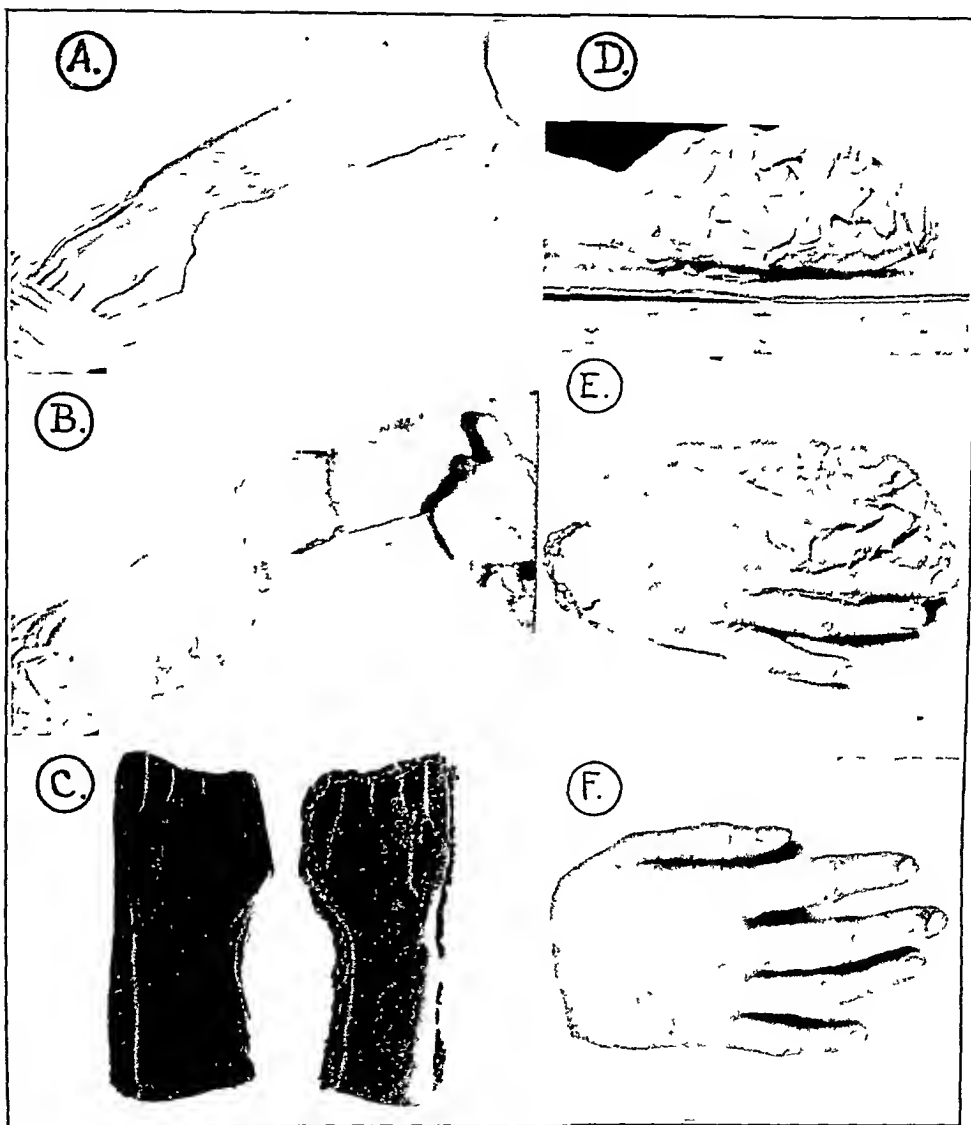


Fig 4—Left, simple plaster mold and cast. Right, simple agar mold and cast. A, The hand and arm are well greased and placed on a cloth. B, They are then covered to a thickness of about a half inch with plaster. A brush is used for the early applications. C, The plaster mold is soaked in water then filled with the resin-wax composition. There are no undercuts in such a mold. D, Greasing the subject is unnecessary when using agar composition. In this case, the hand is covered both above and below with the composition to a thickness of about one inch. After complete setting the hand is slipped out of the mold as one removes a glove. The mold is then filled with resin-wax composition. E, After cooling, the mold is broken away from the cast. F, The cast after removal from the mold. Notice the detail.

Agar and water alone are not suitable for plastic casting, as this mixture is too thin to remain on dependent surfaces and tends to fracture or crumble

too easily. It is necessary for this composition to contain other substances such as preservatives, tanning agents, and materials that will increase the colloidal binding power of the agar, causing the particles to hold together and become more rubber-like. This is the important material for making molds of living and dead tissue.

*The Poller System*—The late Dr. Alphons Poller<sup>3</sup> was evidently the first to recognize the qualities of agar for use in casting and developed his monage system, after which he wrote a book on the subject.

He evidently intended to commercialize his discovery for his compositions were patented, his trade names of "Negocoll," "Dentocoll" (negative compositions), "Granult," "Celerit" and "Hominit" (positive compositions) were registered. Nowhere in his book does he divulge the contents of his compositions. A firm in Switzerland has the rights to manufacture his products which are sold in this country at exorbitant prices. In 1933 there

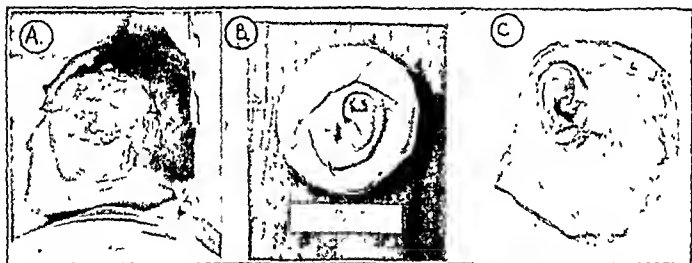


Fig. 5—Casting an ear. A, Small wad of cotton is placed in the deep part of the ear. The agar composition is poured or painted on to the thickness of about one inch. B, The mold is removed, filled with positive composition, colored, and mounted. C, Cast of an ear and tumor of the cheek immediately after removal from the agar mold.

appeared on the market a product called "Form-All" which has the same qualities as negocoll and dentocoll. It is manufactured by an American firm and is materially lower in price than the Poller materials. Their positive material is called "Form-Vex." Another American firm manufactures a negative hydrocolloidal composition which is called "Plastico." The same firm's positive material is known as "Posmonlage."

There are also similar negative compositions called "Surgimould" an elastic compound used by dentists for obtaining impressions of the teeth. In my opinion, all of these molding compositions have an agar base.

In the December issue of *Archives of Pathology*, Volume 16, Number 6, Gross<sup>11</sup> describes a hydrocolloidal composition of his own discovery and gives the following formula and directions for its preparation.

*Preparation of the Negative Mass:* "The negative mass consists of agar,

10. It should be suitable as a mold for the making of plaster of Paris casts.\*

11. If the mold is composed of expensive ingredients, it should be possible to use such material as constitutes the mold over and over again, or the material should be so inexpensive that it can be thrown away after use.

After conducting over a hundred experiments with dozens of different ingredients during a period of three years, I have devised the following formula, which I believe will meet the necessary requirements for making hydrocolloidal molds of living and dead tissue:

Agar	4 ounces
Water	100 ounces
Zinc oxide	1 ounce
Tannic acid	100 grains
Thymol	20 grains
Glycerin	1 ounce
Cellulose	$\frac{1}{2}$ ounce
Cotton	15 grains
Rubber cement	4 ounces

Rubber cement can be purchased from any art, stationery, or five and ten cent store. Such cement generally contains a small percentage of a resin such as damar or mastic. This does no harm to the mass. In fact, Poller includes it in his formulas according to his patents. It can also be made by dissolving a half ounce of caoutchouc (para-rubber) in twenty-five ounces of benzine. This takes about three days.

*Agar* is the basic substance; having the properties, when mixed with water, of melting into a complete liquid with no particles suspended, at about 100° C., and not returning to a gel or setting state until its temperature falls to about 42° C.

The *zinc oxide* increases the colloidal binding power of the agar. Ferric oxide may be used instead, if desired, in place of zinc oxide. Other metallic oxides may also be substituted, subject to experimentation. Zinc oxide causes the resulting mass to be white, while the ferric oxide causes it to be a dark red. In case the zinc oxide is used, the composition may be colored with any alcohol soluble dye to suit the taste of the caster.

The *tannic acid* is a tanning agent and acts somewhat as a separating influence toward gypsum.

The *cellulose* and *cotton* exert a mechanical influence in holding the particles of the mass together, increasing the internal friction of the mass both in the liquid and gel or set state.

The *thymol* and *glycerin* act as preservatives. The glycerin also serves to keep the composition moist. Methyl salicylate is also a good preservative.

\*Unfortunately, a hydrocolloidal composition, such as one containing either agar or glue, is not a suitable molding material for the making of porcelain or terra cotta casts. The reason for this is the fact that these molds do not absorb water from the positive material. Plaster molds, however, do this when the porcelain or terra cotta slip is poured into them. Nor do the agar or glue molds have strength enough to allow the stiffer porcelain or terra cotta pastes to be forced or pressed into place.

The *para-rubber* in the rubber cement is supposed to increase the binding power of the mass and has some effect on its consistency, keeping it from flowing rapidly from a vertical surface

*Directions for Mixing*—Place the agar in about three fourths of the water (75 ounces) (which should be room temperature) in a double boiler and stir vigorously. If the agar is powdered, it mixes readily, however, it should be stirred until all of the lumps have been absorbed. If the agar is in shreds, it will only absorb the water, not going into solution until heated. Heat is applied until the mass goes into complete solution.

The remaining one fourth of the water is placed in a large mouth bottle to which are added the oxide cellulose cotton, thymol, tannic acid, and glycerin. The bottle is corked and the contents are shaken vigorously until they are thoroughly mixed. This shaking in the bottle separates the cellulose and cotton fibers as well as mixes the other ingredients.

After the agar and water have reached  $100^{\circ}\text{C}$ , or the boiling point, and have gone into complete solution, the oxide cellulose or second mixture is poured slowly into the agar or first solution with constant stirring. When these mixed ingredients have again reached  $100^{\circ}\text{C}$ , the mass is removed a safe distance from the fire and the dissolved para rubber or rubber cement, which is very inflammable, is poured slowly in while stirring. The mass bubbles up in evaporating the volatile liquid which was used to dissolve the rubber. It should not be replaced on the fire until the greater percentage of this chemical is removed by evaporation. Otherwise, it will ignite readily. After heating again to about  $100^{\circ}\text{C}$  to drive off any remaining benzine, the mass is poured out into an enamel tray to cool. (If the manufacture of this product is done on a large scale the benzine may be recovered by condensation.) On cooling the composition will set, after which it is ground in a food chopper and allowed to stand until the water has evaporated from it to a point where none can be squeezed from the mass when a ball of it is gripped in the hand. On reheating the mass it is ready for use. Of course, it is allowed to cool to a degree just about setting point before it is applied to living or dead tissue.

The composition is stored in wax covered, air tight containers to prevent drying out. Cylindrical cardboard boxes that have previously been dipped in wax serve well for this purpose.

*Requirements of a Positive Material*—The best positive material for giving a lifelike reproduction of flesh is a composition having a resin and wax base. Plaster papier mâché and similar materials are inferior to wax as far as being "lifelike" for the casting of medical and biologic subjects. The positive composition can be made to be as durable as plaster, providing it is handled with the same care that is given a fine plaster casting.

The resin wax composition used for the positive impression should possess the following qualities

1. It should be possible to brush or "paint" the positive composition into a plaster or agar mold without its cracking or "lifting" from the surface of the mold.

2. Its melting point should be so high that it will not be affected by any degree of heat which is natural in the hottest weather. A cast, no matter how thin, should never droop as a candle often does under normal summer temperatures.

3. It must not crack or shrink to any great extent when subjected to the normal cold of winter.

4. It should not crack under sudden changes of temperature as when a thin layer of hot wax of a high melting point is poured into a cold mold.

5. The shrinkage of the wax should be so slight as not to cause cracks in it when it is shrinking against a rigid mold or after it has been mounted.

6. It should become set, yet remain pliable at a degree of heat low enough to be bent or formed with the hands without breaking or cracking the wax or fear of burning the hands, nor should the composition stick to the hands. This is advantageous as it often becomes necessary to bend slightly a positive impression into a more desirable shape. For example, it may be necessary to bend the positive to relieve it from some undercut surface on a rigid plaster mold. After the positive has been relieved of such an undercut, it can be rebent to its normal position.

Bearing these rigid requirements in mind, let us consider the published material on positive formulas:

Douglas<sup>13</sup> gives the following formula for the preparation of the positive wax impression:

“2 pounds white beeswax  
1¾ pounds paraffin  
1 lb. talcum powder  
1 lb. prepared cornstarch  
2 oz. yellow beeswax”

The talcum powder and cornstarch used in this formula do not increase the melting point of the combined waxes which is dangerously low (see table of waxes). While the cornstarch and talcum powder will decrease the cost and increase the bulk of the composition, I do not advise its use because it definitely increases the possibility of breakage or of crumbling which is alleviated somewhat by the low melting point and high cohesive qualities of the combined waxes used. (Had stearic acid been used, the possibility of crumbling would have been increased, due to the crystalline structure of this substance and its low cohesive qualities.)

It seems to me that the yellow beeswax is used in this formula more for the purpose of coloring than anything else. However, it may increase the pliability of the composition very slightly. If it is used for coloring only, it seems unnecessary, as pigments have to be added anyway to bring the wax to the desired color or shade. This formula cannot be brushed or painted into the mold successfully.

In a mimeograph sheet, issued by D. E. Ziskin,<sup>14</sup> of the School of Dental and Oral Surgery at Columbia University, the following formula is given:

"11 ounces white filtered paraffin (high fusing)  
 2 ounces light carnauba wax  
 1/4 ounce beeswax  
 1/4 ounce dark carnauba wax"

This formula has a higher melting point than that of Douglas<sup>13</sup> and for this reason, I consider it more valuable. However, when we consider the three basic properties of each wax, i.e., expansion on heating, contraction on cooling, and elasticity, we find that carnauba has high cohesive and low adhesive properties. Therefore, it should be used only as a hardener and to raise the melting point out of danger of the heat of summer. For the above mentioned reasons, Zishin's formula is very likely to crack when poured into a cold mold or subjected to violent changes in temperature. This could have been prevented by the addition of a resinous gum which would also increase the melting point. This formula cannot be painted into the mold successfully.

It seems that the small amount of dark carnauba wax was used mainly for coloring the composition, as it has the same melting point as the light carnauba. The same may be said of the beeswax providing it is yellow beeswax, for the quantities are so small that they change the cohesive and adhesive qualities to a minor degree. I believe in relying mainly on pigment for coloring rather than using dark waxes for this purpose, which do little more than complicate the formula.

In Golden's article<sup>7</sup> which has been mentioned before under wax molds, he also considers positive compositions. For the wax mold, he naturally writes of substances which can be used without the application of heat. One of these is "a high grade of plaster having the advantages of ordinary plaster as to convenience in setting time and greater hardness than the artificial stones heretofore used. This plaster is known by the trade name of orthopedic plaster, or Hydriocel 'B' ". It is made by calcinating dry gypsum rock under steam pressure which gives larger crystal structure than possible with ordinary plaster.<sup>15</sup> The normal setting time of this material ranges from twenty five to fifty minutes. However setting can be accelerated to a few minutes or retarded for several hours. As mentioned before a resin wax mixture gives a more lifelike reproduction.

Golden also considers "hominit and the other Pollet positive materials". Of "hominit" he writes as follows:

"According to analytic tests the product consists of Carnauba wax, 73 per cent, rosin 3 per cent, ash (composed of silica lime and magnesia), 24 per cent.

"From the tests appearance and color no doubt a medium grade of No 1 North country carnauba wax is used<sup>16</sup> this giving the compound a brownish gray appearance due to impurities in this grade of wax. This color does not lend itself very readily for tinting and its dark appearance if not tinted, is deceptive in taking casts of the white race.

"The mixture could be benefited greatly by the use of No 1 Florida pale yellow carnauba wax which is the highest grade. By using this grade of



wax, a very light appearance should be assured which would allow the use of various tints to a finer degree, to give a natural lifelike skin coloring to the finished cast.

"By the addition of a small percentage of paraffin wax, the higher temperature wax preferably, ranging from 5 to 15 per cent, a milky tinge is given to the compound; which is an advantage in making death masks. This cloudy appearance can also be taken advantage of in the making of casts of children and women."

I disagree with Golden about the contents of "hominit" due to its very nature. The addition of only 3 per cent of a resin would not give the mass its characteristic bending qualities; nor would it be possible to brush the material into the mold. Furthermore, if it contained the amount of carnauba wax that Golden specifies, the mass would readily shrink and crack. Poller, himself, says in his British patent specifications:<sup>17</sup> "The colophony will always amount to more than half the resin mixture as otherwise the treatment with the brush is rendered more difficult." Poller continues to state that the carnauba should not exceed one-tenth of the amount of resin. He gives the following proportions:

"Resin	14
Paraffin	2
(Clear) Carnauba	1"

I believe that these proportions are misleading in the fact that the formula may overestimate the amount of resin that is actually used in the average composition. It does not necessarily over- or understate the amount of carnauba wax used. I also believe that the amount of paraffin is underestimated in Poller's formula. As stated before: this is often done in patents to prevent commercialization.

In spite of the fact that Golden recommends the use of No. 1 Flora pale yellow carnauba wax and 5 to 15 per cent of high temperature paraffin wax, the composition is still very likely to be too yellow for the normal Caucasian skin. This can be eliminated by the use of a filler or color desensitizer such as talc, plaster, or kaolin (see author's formula). Any formula containing 73 per cent of carnauba wax will give a compound sure to shrink and crack on being poured in thin layers into a cold mold. It would be impossible to obtain a good cast by brushing a composition of this nature into any kind of mold. I do not believe that a really successful formula, which will meet the rigid requirements set down in this text, can be compounded from Golden's analysis of "hominit."

In further consideration of Poller's positive materials, such as "hominit," "eelerit," and "granulit," there seems to be little difference in them other than color and melting points. Waxes and resins may be obtained in various shades of brown and yellow. These shades may be changed by the use of pigments, fillers, and color desensitizers. If one consults the melting points in the table of waxes and the table of resins, to say nothing of the facts laid down in this article under "*Compounding the Positive Formula*," one can

easily determine the materials to use to raise or lower the melting point of a composition. In such a manner the worker may compound formulas for specific purposes.

I believe that the major reason for producing positive compositions under different names lies in their commercial possibilities. I have yet to discover the advantages of starting out by using "hommit" for a thin coating to the mold, and then switching over to "eclerit" to back up the cast or thicken it as Poller recommends. If one prepares his own mixture of a sufficiently high melting point and of the right color, he can use this one mixture for the entire thickness of his cast. Of course, some cloth or fiber material is used to strengthen the positive.

Toward the end of the article by Gross,<sup>10</sup> in the *Archives of Pathology*, mentioned before in this article, when we considered negative formulas, he recommends the use of plaster of Paris, sealing wax or paraffin for making the positive impression. I believe that there are far more suitable compositions for making the positive than any of the three that Gross recommends. The reasons for this belief are as follows. It is extremely difficult to obtain

TABLE I  
TABLE OF WAXES

NAME	MELTING POINT	COLOR	SOURCE
Bayberry	45°-46° C 113°-114.8° F	Light green	Vegetable wax
Beeswax (natural) Apis mellifera	62°-64° C 143.6°-147.2° F	Yellowish brown	Insect wax
Beeswax (bleached) Apis mellifera	62°-64° C 143.6°-147.2° F	White	Insect wax
Candelilla	68°-70° C 154°-158° F	Brownish to yellowish brown	Vegetable wax
Carnauba (light) Corypha cerifera	84° C 183.2° F	Light yellow	Vegetable non glyceridic
Ceresin (ozokernite light)	61°-78° C 142°-172° F	White	Mineral wax
Ceresin (ozokernite dark)	61°-78° C 142°-172° F	Light yellow	Mineral wax
Chinese (Cocos cerifera)	About 81° C 172° F	Light yellow	Insect wax
Japan Rhus succedaneum	49° C 120° F	Light yellow	Vegetable fat
Montan	90°-86° C 176°-187° F	Dark brown to white when bleached	
Paraffin*	48°-62° C 118°-144° F	White semitransparent	Mineral wax
Parawax†	51°-58° C 124°-137° F	White semitransparent	Mineral wax
Spermaceti	42°-50° C 107°-122° F	Pearly white becomes yellow on prolonged exposure	Sperm whale wax
Stearic Acid (Stearine)	60°-67° C 140°-149° F	White crystalline structure	Chemical

\*The melting point of paraffin varies from 18°C to 62°C. It can be purchased for melting at the following temperatures: 18° to 50°, 50° to 52°, 52° to 54°, 56° to 58°, 60° to 62° C.

†Parawax is a trade name for paraffin manufactured by the Standard Oil Company of New Jersey. This wax is cheaper than some paraffins which melt at specific temperatures and serves just as well for the purposes of wax casting.

a truly lifelike positive in plaster. Gypsum has a tendency to crumble, or "rot" as it is termed by casters, when incorporated with certain pigments. It does not have the texture or semi-translucency of skin or flesh as do wax and resin. In coloring the plaster of Paris positive, the color seems to lie on the surface rather than actually to appear as though it were incorporated in the cast.

TABLE II  
TABLE OF RESINS

	PROPERTIES	SPECIFIC GRAVITY	MELTING POINT	SOLUBILITY
Asphaltum	Black or brownish black substance intense in chromatic value	1.0 to 1.68	100° C.	Five times its weight of naphtha and benzol Dissolved by alkalis and alkaline carbonates
Colophony or common rosin	Brittle, transparent pale amber to dark red brown	1.045 to 1.085	Softens at 70° C. to 80° C. semi-fluid in boiling water. 135° C. should not be exceeded	Methyl and amyl alcohol, acetone, ether, chloroform, carbon disulphide. Partial—at 60° C. dissolves slowly in equal weight of alcohol or glacial acetic acid. Petroleum spirit
Copal	White through shades of yellow, and red to brown and brownish black. Fossil resins have an oxidized crust containing mineral matter. Tree resin is odorless	1.03 to 1.07	95° C. to 305° C.	95% alcohol, oxidized turpentine, alkaline solutions
Dammar	Nodules—3 mm. to 4 em. Powdery crust. Clear. Transparent. Odorless	1.062 to 1.23	Softens at 75° C. Syrupy at 100° C. Clear and thin at 150° C.	Benzol, chloroform, carbon disulphide, oil of turpentine Partial: Methyl and ethyl alcohols, ether, acetone, glacial acetic acid
Mastic	Grains ovoid in shape. 0.5 em. to 2 em. Milky, dull yellow or greenish. Taste and smell like turnips	1.04 to 1.07	Chios 108° C. Other 103° C.	80% chloral hydrate solution, ether, amyl alcohol, benzine Partial: Methyl alcohol, acetone, acetic acid, chloroform, oil of turpentine
Sandarac	Form of grains 0.5 to 1.5 em. Clear yellow drops. Surface has dusty appearance. Harder than mastic	Between 1.05 and 1.092	Liquefies at 160° C.	Alcohol, ether, amyl alcohol, acetone, and several ethereal oils Partial: Chloroform, turpentine. Fatty oils
Shellac	Thin, translucent hard flakes. Varies in color from yellowish brown to black. Bleached or white shellac: Prepared by dissolving crude lac in potash of soda, filtering and passing chlorine gas into the filtrate till all is precipitated	1.139		Methyl and ethyl alcohols, acetic acid, alkalis, solution of borax Partial: Ether, ethyl acetate, chloroform, acetone, carbon disulphide

Sealing wax is expensive to buy or to prepare in comparison to other compositions. Furthermore its internal friction is too great for convenient use. In other words, it flows too sluggishly. It also has too much "gloss" for some cases. It is better to apply the "gloss" in the form of shellac or varnish to the area in mind than to try to remove the glossiness from the area where it is not wanted.

Paraffin, when used alone has a melting point that is so low that the positive is likely to be destroyed by the heat of summer. It also has a high degree of gloss and is semi-transparent. I do not recommend the use of any of the substances in the form as mentioned by Gross for the making of casts of medical subjects.

In *Colorado Medicine*, there appeared an article by Mumey<sup>18</sup> of Denver. This article recommends the use of plaster of Paris for making the molds of surgical specimens. This I do not advise. He also calls attention to the hydrocolloidal formula developed by Gross.

For his positive formula this author gives the following:

White beeswax	8 ounces
Paraffin (high melting point 6° to 58°)	8 ounces
Cornstarch	6 ounces
Talcum powder	6 ounces
Spermaceti	1 ounce

(Yellow beeswax may be added for color)

"The wax and paraffin are melted before adding the starch and powder."

In my opinion this formula seems of little importance because of its low melting point and the fact that there is little difference in his three waxes (see table of waxes). Any one of them could have taken the place of all three without any material loss to the composition. Talcum powder and cornstarch in such proportions act as little more than fillers, cheapening the product and at the same time making it more crumbly.

It is interesting to notice that this author says "The inside of the mold is coated with liquid albolene which aids in separating the wax positive from the plaster negative." I have never heard of this practice before in separating wax from plaster as most casters simply dip the plaster negative in water before "painting" or pouring the positive composition into it. The antagonistic qualities or immiscibility of oil (wax) and water create a perfect separating medium.

He also continues "The positive wax cast is removed from the negative plaster cast by making saw cuts into the negative and carefully breaking it loose with a hammer." In my estimation this is indeed a hazardous and clumsy method of casting in consideration of the fact that Mumey was familiar with the hydrocolloidal formula of Gross and the commercial product "negocoll." His formula cannot be "painted" into the mold.

In considering, as a whole the positive formulas which have been mentioned in this article and published before, the following facts are evident:

I. They do not contain resin (except Poller's formula, in which I believe

the amount of resin is overestimated, and Golden's analysis of Poller's "hominit" which I believe is incorrect in the sense that the resin is underestimated).

2. They cannot be brushed successfully into the mold. (The only exception that may be to this is the formula of Poller.)

3. These formulas often contain more than one wax of about the same melting points, which I believe is unnecessary.

4. They often contain a small amount of an intense yellow or brownish yellow wax as a coloring agent. Color, I believe, should be applied to the positive formula in the form of color pigments.

5. Their melting points are dangerously low. I believe that the melting points should be higher.

It is for the reasons given above that I do not recommend the use of any of the formulas that are mentioned in this article, other than that humbly submitted by myself.

#### ADVANTAGES OF BEING ABLE TO APPLY THE POSITIVE MATERIAL WITH A BRUSH

A positive resin-wax material which permits itself to be spread, or painted, instead of poured, into the mold offers decided advantages which may be enumerated as follows:

1. It eliminates the danger of the much dreaded borderlines, i.e., lines caused by the junction of hot and cold wax on a smooth surface. The hot and cold wax do not blend together, thereby forming a line where the waxes of different temperatures meet (Fig. 6-A).

2. The painting on of the positive permits a freeness of working without the necessity of having to lift heavy molds to turn them in various positions so the wax will flow evenly and of the same thickness over the entire mold. When large forms are to be poured, it otherwise would require a great outlay of physical strength and nerve-racking exertion to say nothing of the necessity of the assistance of helpmates to produce the cast.

3. It is far more economical to "paint" a mold than to pour one, as considerable wax may be lost or damaged beyond further use by slopping over the edges of the mold while it is being rotated, thereby soiling one's clothes and burning the hands. The composition may fall on the floor, worktable, or anywhere but in the place it should be.

4. It is possible to fill the separate pieces of a piece mold before they are put in place to insure their surfaces of being well and evenly covered. After they have been put in their proper places, the positive pieces may be cemented together by the application of more wax which may be poured, or painted, into the cracks between the positive pieces.

5. In painting with the positive substance rather than pouring it into the mold, it is possible to apply masses of different materials and colors next to one another in the same layer, or different colors in different layers, thereby producing the transparency of one color through which a second color may be seen beneath the first. One may spread on the mold a wax composition to resemble the skin in color and texture to the edges of a gaping,

moist, reddish wound that would ordinarily be glistening with high lights. This wound area of the cast can be painted in with a different material and color than that of the skin. While this second material may have a wax base, it can be so incorporated with other materials such as various gums to produce the desired glossy effect.

6 One may prepare a palette of an ordinary muffin pan, filling each cup with the positive wax material with which has been mixed a different color. The caster may then use this muffin pan as an artist does his palette in painting a canvas, the caster paints his mold instead. The thin applications of

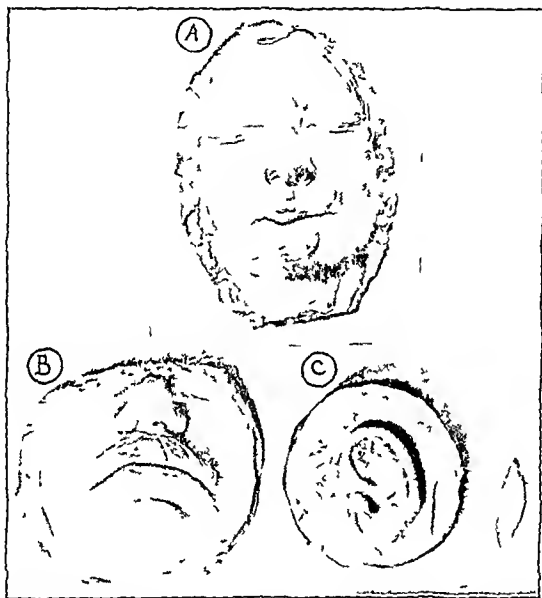


Fig. 6—Mistakes of casting. A Border or lap lines caused by an improper mixture of positive material being poured slowly into a cold mold. These lines are due to the fact that one layer of wax has set before an adjoining layer is poured. B Cracks caused by shrinkage due to improper mixture of positive materials. Such cracks are caused by too much carnauba wax or not enough resin in the composition. C Showing that gauze reinforcement was not completely saturated with hot wax before application. All fibrous materials should be dipped in hot wax before placing in the mold.

the various colors on this mold must be strengthened or backed up by a thicker layer of wax, then reinforced with gauze, cheesecloth or burlap fibers, or threads.

In such a manner, the small blue vessels that lie beneath the transparent skin can be incorporated into the cast in the most naturalistic manner. An other example for the use of color in this way is a typical hemorrhage under the skin. The fresh flowing blood possesses a different color from stagnant

blood. In such a manner vessels may be painted red on a thin coating of semi-transparent positive formula which has been applied to the mold. These vessel details are then backed with more composition and finally strengthened with gauze, cheesecloth, or burlap. When the cast is finally removed from the mold, the vessels may be seen as though they lie under the skin rather

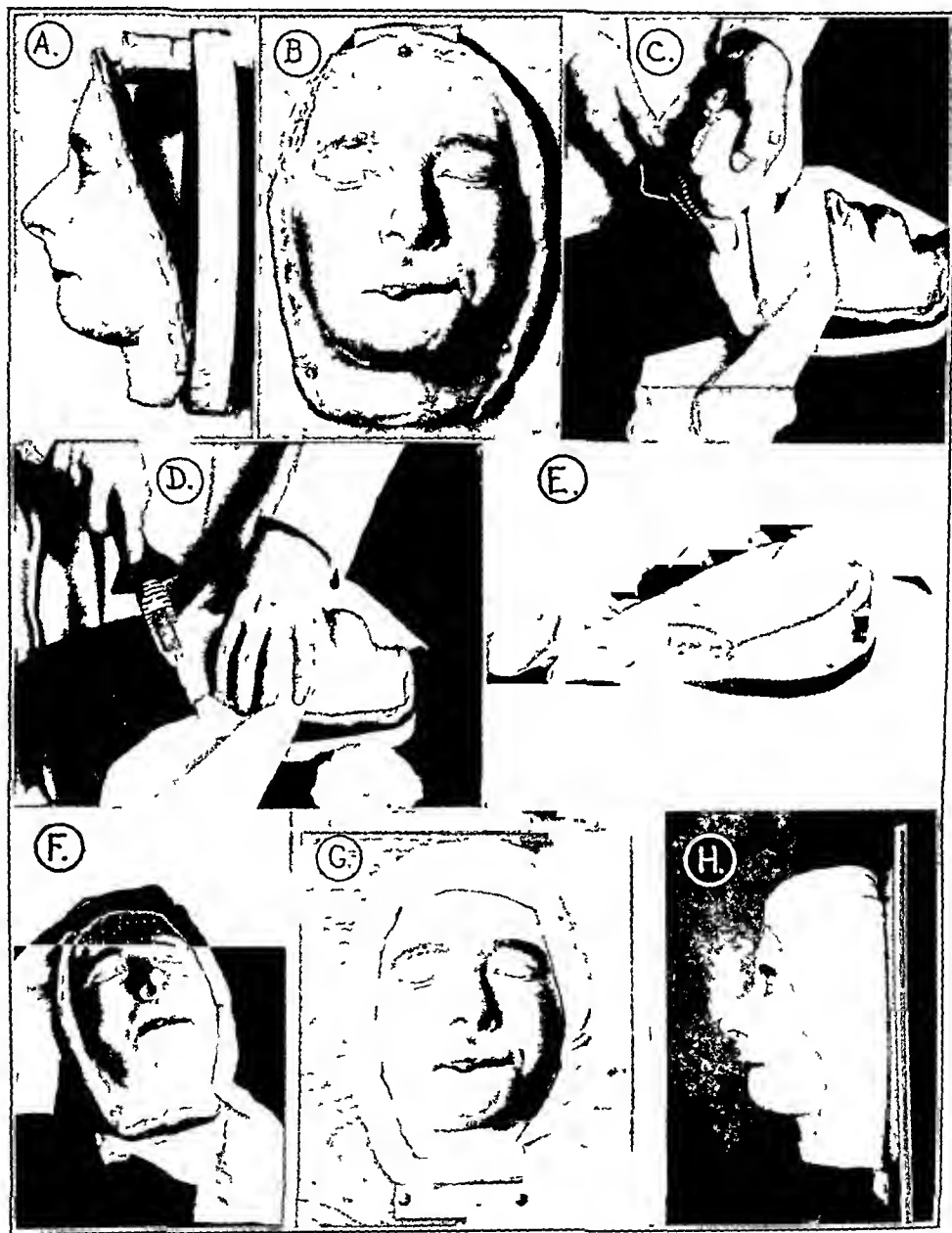


Fig 7.—Mounting. A, Side view of cast on submount. B, Notice that the submount takes the outline of the cast. C, Gauze is sealed to the edges of the cast which is applied with a hot electric tacking iron. D, The iron is used to seal the edges flat. E, This is continued all around the edges. Pleats are made where necessary. F, The gauze is finally turned under the submount and attached with carpet tacks. G, The submount is then screwed to the mount. H, Side view of the cast on the mount.

than painted on the surface as is so often done by the caster. It is interesting to note that red vessels under an ivory colored skin appear blue when viewed through the skin. This can be tested by placing a piece of ivory colored tracing paper over dark red marks drawn on white paper. These red marks will seem to be blue providing the right shade of tracing paper and the proper shade of red are used.

There are limitless possibilities for the specific use of a positive material that can be painted into the mold rather than having to be poured. In fact, they are too numerous to continue to mention here. However, they will suggest themselves as the worker begins the making of molds and casts.

*Compounding the Positive Formulas*—Before preparing formulas for positive impressions bear in mind that every substance in them must be there for a definite purpose. It seems that most casters mix a number of waxes together with great quantities of starch and a filler such as talc with little knowledge of the properties of the substances they use. White waxes having approximately the same melting points are likely to react in much the same way. Using more than one of these only complicates the formula and fools no one but the novice. Do not necessarily use a yellow wax for color, this is the work for color pigments to do which have a far greater range of shades and tones than waxes. Furthermore pigments are more dependable. In the best formulas it is often necessary to reduce the chromatic intensity of some waxes and resins rather than to add yellow wax to give them color. The following data are essential for the worker to bear in mind when he compounds formulas.

*Resin* In spite of the fact that such work is often called "wax" casting, resin is the basic substance in the formulas that are the most simple to use and have most versatility. It gives the composition toughness and the quality of bending without breaking hardens more slowly and prevents warping and cracking to a great extent. Common rosin or colophony is generally used for this purpose because of its cheapness. Bleached rosin serves better where the chromatic intensity is to be kept low. The resin content should amount to more than half of the mixture, otherwise brushing the positive mixture into the mold is rendered difficult, leaving "lap lines" or borderlines. Such lines are caused where a hot mixture meets a cold or set mixture of the positive material, causing a line to appear at the junction of the hot and cold mixtures (Fig 6 A).

*Balsams and Amylum* Brittleness of resins can be reduced by the addition of balsams and amyllum. The following balsams may be considered: Copiba, Canada, Peruvian balsam, and gum elemi. As for the amyllum, cornstarch and more particularly wheat starch may be considered. These starches tend to lower the chromatic intensity of the resin. Only a small quantity, about one tenth of the weight of the resin, should be used. Balsams lower the melting point of the positive formulas. Amyllum does not lower the melting point.

*Wax (Low Melting Point)* The viscosity, and viscosity, or internal friction, of a resin is reduced by the addition of a wax. A cheap wax, necessarily having a low melting point, is chosen for this purpose. Such waxes are "paraffin," paraffin white beeswax, ceresin, and spermacetic. Here again the cheaper products ("paraffin" and paraffin) are generally chosen.

*Wax (High Melting Point)* The melting point of a wax and resin composition can be raised by the addition of a wax having a high melting point. Such waxes are carnauba, montan, and Chinese. Carnauba (light) is generally chosen. The amount of such a wax that is used in the composition should never be more than one tenth of the weight of the resin, otherwise shrinkage and brittleness of the entire composition will result.



**Zinc Oxide:** Zinc oxide may be used to render the resin harder and less sticky, also giving the composition a higher melting point. The weight of the zinc oxide should never be more than one-thirty-fifth of the weight of the resin. If more is added, a frothing up occurs and the mass will not remelt once it has set. It also will become as hard as stone, and crumbly.

**Fillers, Toners and Color Descensitizers:** Substances such as talc, plaster of Paris, kaolin, marble meal, whiting, powdered glass, and silver and gold or metallic powders have little effect on the melting point of positive resinous masses and serve only as fillers and to change the shade or color of the resulting compositions. *If such substances are added in too great quantities, the composition becomes brittle and crumbly.* Some white fillers, such as talc and plaster, are excellent for reducing the chromatic intensity of resins.

**Volatile or Drying Oils and Hydrocarbons:** Hydrocarbons such as benzine and drying oils such as turpentine, linseed, poppy oil, and naphtha may be used to lower, temporarily, the melting point of the mass (especially of hard waxes). This helps the resin to facilitate the bending of the positive impression after cooling, for removing it from undercuts in the rigid plaster negative. A few drops of benzine or some volatile oils eliminate the slight froth caused by the addition of zinc oxide to a formula. This slight addition does not harm the mass materially by lowering the melting point. The pot containing the composition should be moved a safe distance from the fire before adding such inflammable liquids. Continuous heating of the same positive mass is likely to raise the melting point and cause it to become brittle. This can be given its original properties by the addition of a small amount of drying oil. Some casters prefer to remove the cast from the mold after it has completely cooled. In such a case the addition of a drying oil is advantageous. However, it is often better to remove a cast, having a resin base from the mold when it is still slightly warm and pliable. In such a case the addition of a liquid to lower, temporarily, the melting point is unnecessary.

**Binders:** Casts may be strengthened by the use of cotton, hemp fibers, cheesecloth, gauze bandages, wool, burlap, and similar substances. Such materials are generally dipped in the hot positive composition and applied to the back of the cast after it has reached the thickness of about an eighth of an inch. These binders are used whether the positive is brushed or poured into the mold. This, of course, is done while the cast is still in the mold. According to Poller, "Northern" or Harras wool may be mixed directly with the positive formula for the purpose of binding the particles to one another. Such fibers do not "lump" together. If the cast is well supported with one or more of these binders, the chances of its being completely demolished on dropping a short distance to the floor is greatly reduced. In many cases, repairs are easily accomplished.

**The Author's Positive Formula.**—A good, simple and cheap (less than fifteen cents per pound) formula that I have devised to meet the rigid requirements set down further back in this text is as follows: This formula can be brushed or "painted" into the plaster or agar mold. It may be varied to suit specific requirements. "Parawax" is given before paraffin because it is cheaper. If a paraffin is used, choose one of a melting point similar to "parawax" (about 51° C. to 58° C.).

"Parawax" (or paraffin)	5 ounces
Rosin (colophony, bleached)	10 ounces
Carnauba wax (light)	1 ounce
Talc	4 ounces
Zinc oxide	½ ounce

(Color to suit hue of subject)\*

\*Roehrig's transparent oil colors (for photo-tinting). If this is to be the skin color of a caucasian, it is only necessary to add a red pigment such as vermillion (blond) or rose madder (brunette). The red mixes with the yellow of the resin and carnauba, giving a "flesh tint."

The paraffin and carnauba wax are melted together until they are both in a liquid state. To prevent burning a double boiler is generally used for this purpose. The resin is then added and the composition stirred until the resin mixes with the wax into a homogeneous mass. The tale, which is used to reduce the chromatic intensity of the resin, is added with constant stirring, and then the zinc oxide while the stirring is continued. When the zinc oxide is added, a slight froth or foam may collect on the surface of the mixture. This may be expelled with a few drops of benzine which should not be added until the melting pot is a safe distance from the fire.

Color is added by placing a small bit in a tablespoon then filling the spoon with some of the melted composition. The bottom of the bowl of a teaspoon is used to mix the color into the small quantity of wax in the tablespoon. After the color is well mixed in the tablespoon, both spoons are used to stir the pot of composition thus permeating it with color. This process is gone through with because the color when placed directly into the composition, does not mix readily with it. Bear in mind that color should be used sparingly because it is difficult to reduce the color intensity by the addition of a filler without harming the entire structure of the positive mass. Wax-resin compositions decrease in chromo value when the mass cools. If it is so desired, this formula can be mixed as a medium in small quantities with many shades and colors. The caster can paint the inside of his mold with the color he desires. Of course this does not mean that more color should not be added to the cast after removal from the mold. In fact it should be touched up with oil tempera or water colors to make it more realistic.

Let us consider why certain substances were used in the formula that has just been given.

1 The "parawax" or paraffin was used to reduce the internal friction or sluggishness of the resin base.

2 The resin among other reasons mentioned before made it possible to "paint" or brush the composition into the mold using any small paint or varnish brush for the purpose.

3 The carnauba wax raised the melting point out of danger of summer heat.

4 The tale reduced the chromatic intensity of the yellow of the resin and carnauba thereby making it possible to obtain a true flesh tint in the entire composition.

5 The zinc oxide reduced the stickiness of the resin and helped raise the melting point of the completely cooled mass.

6 The benzine eliminated the foam, thereby keeping it from appearing on the surface. This is not always necessary but is good insurance against a bubble infested surface on the cast.

This formula may be varied to suit particular purposes and climates. For example. In cold climates the amount of carnauba wax may be reduced. In hot climates it may be increased. Different fillers may be used to create the effect of metal, wood, stone and marble, according to what is desired.

A formula better suitable for pouring should contain more wax and less resin than the formula given above as the internal friction of the above formula is too great.

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## AN IMPROVED CONGO RED TEST FOR AMYLOIDOSIS\*

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KEITH, Rowntree and Geraghty<sup>1</sup> introduced a method for the determination of blood volume by means of vital red. This dye was injected intravenously and normally did not leave the blood stream in four to six minutes. By that time the dye was found to be distributed evenly in the circulating blood. Keith and others have shown that the dye does not enter the blood cells and can be found quantitatively in the serum.

Griesbach,<sup>2</sup> in his study of blood volume injected 10 cc. of a 1 per cent Congo red solution and found a uniform distribution of the dye in the serum after four minutes. The dye began to leave the blood ten minutes after injection, however, after one hour 90 per cent was still in the circulation of normal subjects, and after twenty-four hours no further traces of Congo red could be found in the serum.

Bennhold<sup>3</sup> found that in persons with amyloid tissues Congo red leaves the blood stream either in toto or in large part within an hour. His method was to inject intravenously 10 cc. of a 1 per cent Congo red solution and withdraw two specimens of blood the first at four minutes and the next one hour after injection. The blood was then centrifuged and comparison made on the serum by means of the colorimeter. The four minute specimen was used as the standard from which the amount of absorption in one hour was determined. Using this technique he found a disappearance of 11 to 29 per cent of the dye from the blood stream of normal persons in one hour whereas if 60 per cent or more of the dye disappeared amyloidosis was indicated. When the absorption fell between 40 and 60 per cent, he considered it indicative either of nephrosis or amyloidosis.

The Congo red test for amyloidosis used in the past is satisfactory, providing the serum obtained is unhemolyzed. Extreme precautions are necessary, otherwise the clinician can rely on values only where the absorption from the blood stream is complete, and even with these precautions there can be no assurance that hemolysis has not occurred unless the specimen is examined with the spectroscope. An excessive amount of lipoids in the serum gives a turbidity that interferes with colorimetric readings.

These disadvantages can be overcome by the use of ethyl alcohol which precipitates the proteins and dissolves the Congo red giving a clear solution that can be read with the colorimeter.

*Method*—The test is carried out on the patient in a postabsorptive state. Ten cubic centimeters of a 1 per cent Congo red solution are injected into a vein and four minutes later about 10 cc. of blood are withdrawn from another vein. The second sample of blood is taken

\*From the Laboratory Division, Sea View Hospital.  
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one hour after injection. Here it might be advisable for the tubes to stand about two hours to permit the clots to retract. The tubes are next centrifuged at a moderate speed (3,000 r.p.m.) for ten minutes, and the serum carefully decanted. Two cubic centimeters of serum are pipetted into 15 c.c. graduated conical test tubes and made up to 10 c.c. with 95 per cent ethyl alcohol. The tubes are corked, shaken for thirty seconds, and centrifuged at a high speed for ten minutes. (Absolute alcohol may be used, although it is not essential.) The clear supernatant fluids are poured into colorimeter cups and readings made on the colorimeter. The readings are best when the four-minute specimen is set at 20 mm. The calculation is

$$100 - \left( \frac{4 \text{ min. reading}}{1 \text{ hour reading}} \times 100 \right) = \text{per cent absorption.}$$

The solution remains unchanged indefinitely when corked. Lipoidal serum gives a very clear supernatant alcoholic solution.

Any hemoglobin that may be present is precipitated by the alcohol, and the Congo red determined quantitatively. Readings before and after alcohol precipitation checked very closely, except where hemolysis was known to occur. In the table below can be seen comparisons of the same specimens by both methods:

TABLE I

SERUM READ DIRECTLY PER CENT ABSORPTION	AFTER ALCOHOL PRECIPITATION PER CENT ABSORPTION	DIFFERENCE PER CENT
11	12	+1
15	12	-3
36	30	-6
10	10	0
31	33	+2

This method can be applied only to serum. Experiments on whole blood with added Congo red do not give quantitative recoveries when the dye is extracted with alcohol. It seems that some Congo red is carried down with the precipitated hemoglobin, which if present in small amounts in the serum does not affect the readings. Congo red added to serum is extracted quantitatively with alcohol.

The test has been satisfactorily applied in the authors' laboratory. One case is especially of interest. A patient was admitted into the hospital with a recent history of amyloidosis. He still showed clinical signs of the disease, a four-plus albumin, and Congo red in the urine after administration of the dye. Examination of the serum directly showed no absorption, in fact the one-hour specimen was slightly stronger in color than the one of four minutes. The addition of alcohol to the serum showed the absorption to be complete. Through use of this method it was possible to correlate the laboratory and clinical findings.

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# IMPROVED PROCEDURES FOR ESTIMATING BISMUTH IN BODY FLUIDS AND TISSUES\*

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THE demand for reliable methods for the quantitative estimation of minute quantities of bismuth which may exist in body fluids and tissues, has increased since the advent of bismuth in antisypilitic treatment. Experiences in this laboratory during a period of eight years with a method, which has been steadily improved and simplified, have resulted in routine procedures which may be of interest and value to others. These procedures are applicable to all body fluids and tissues and have been used successfully in thousands of estimations. They are based upon unpublished data and some published data scattered through several publications from this laboratory. Many details, included in the original description,<sup>1</sup> are omitted here as they are no longer essential for most users of bismuth methods. The essential technical details and steps including some typical results, follow.

**Equipment**—Kjeldahl flasks (Pyrex) 300 cc. Folin sugar tubes (Pyrex), calibrated to 4 cc and 25 cc, two 500 cc burets one for  $\text{HNO}_3$  and one for  $\text{H}_2\text{SO}_4$ , two 200 cc volumetric flasks for sulphate and iodide solutions fitted with 5 cc pipettes. Dubosecq colorimeter, gas hot plates, system of Pyrex glass manifolds and water tower connected with a suction device for the removal of fumes from the Kjeldahl flasks, 30 of which are connected in 3 groups of 10 each. crucibles (porcelain or silica) capacity 50 cc electric furnace (Multiple Electric Unit Furnace Type 66 No 39 461, New Duty Electric Co.), steam or water bath.

**Solutions**—*Standard bismuth solution* (Leonard) 0.223 gm  $\text{Bi}_2\text{O}_3$  (analyzed CP, and dried to constant weight at  $110^\circ$  ) is dissolved in a small amount of  $\text{HNO}_3$  and made up to 2,000 cc, 1 cc of solution = 0.1 mg Bismuth. The standards for 0.001, 0.002, 0.005 and 0.01 mg are set at 90 mm on the colorimeter, the standard for 0.02 mg is set at 50 mm (using 20 cc cup) the standard for 0.05 mg is set at 30 mm, the standards for 0.1 and 0.2 mg are set at 20 mm. *Potassium iodide solution* 2.4 gm of chemically pure (analyzed) KI are dissolved in 200 cc distilled water, 5 cc of this solution are used in each determination. The solution is discarded if it becomes discolored. *Acidified sodium sulphite solution* 1 gm ( $\text{Na}_2\text{SO}_3$ ) is dissolved in 150 cc distilled water, then 0.8 cc concentrated  $\text{H}_2\text{SO}_4$  are added and sufficient water to make 200 cc. 1 cc of this solution are used in each determination. This solution must be prepared fresh daily. *Superoxol* (concentrated  $\text{H}_2\text{O}_2$ ) concentrated sulphuric acid (P) concentrated nitric acid, (P), amylalcohol,  $\text{KNO}_3$  (dry CP, analyzed) *Permanent standards*

0.5000 gm potassium chromate (CP) in 1500 cc water	= 0.20 mg Bi
0.0750 gm potassium chromate (CP) in 490 cc water	= 0.100 mg Bi
0.7800 gm potassium chromate (CP) in 1000 cc water	= 0.050 mg Bi
0.0500 gm potassium chromate (CP) in 1500 cc water	= 0.020 mg Bi
0.0174 gm potassium chromate (CP) in 1,000 cc water	= 0.010 mg Bi
0.0077 gm potassium chromate (CP) in 1,000 cc water	= 0.005 mg Bi
0.0066 gm potassium chromate (CP) in 1,000 cc water	= 0.002 mg Bi
0.0064 gm potassium chromate (CP) in 1,000 cc water	= 0.001 mg Bi

\*From the Department of Pharmacology, Stanford University School of Medicine.  
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*Check of permanent standards:* Introduce 10 c.c. of distilled water and add 5 c.c. of concentrated sulphuric acid each into four 25 c.c. calibrated sugar tubes. Add enough of the standard bismuth solution to contain 0.2, 0.1, 0.05, and 0.02 mg. of bismuth in different tubes. Cool the tubes and make up to the 25 c.c. mark, using 4 c.c. of sulphite water. Now add 5 c.c. of potassium iodide solution and match the permanent standards.

*Urine.*—The volume of urine to be used depends on the bismuth content. A 50 c.c. aliquot portion of a 24-hour sample is generally used. Human and animal (rabbit) urines are treated the same. To the urine contained in a Kjeldahl flask add a drop or two of caprylic alcohol, 5 c.c. of concentrated sulphuric acid, 10 c.c. of concentrated nitric acid, and 5 c.c. of superoxol. The flask is connected with the system of manifolds with a loose gooseneck tube, and combustion is started at once.

Boil on the hot plate until the nitric acid fumes have disappeared and the organic material is charred. Allow to cool partially and then further additions of nitric acid may be made without sputtering. If the acid (about 5 c.c.) is carefully added, frequent additions may be made even while the flask is on the hot plate. Superoxol is also added in the same way from time to time until the residue is water-clear. The removal of the last traces of oxides of nitrogen, which, by liberation of iodine from potassium iodide, affect the color of the determination, is achieved by adding 2 or 3 c.c. of superoxol to the residue and heating until the white fumes of  $\text{SO}_2$  are formed.

*Colorimetric Estimation.*—Transfer the residue, which should be at least 4 c.c., from the Kjeldahl flask to a 25 c.c. Folin sugar tube, using a few small rinsings of water so as to bring the total volume in the tube to approximately 21 c.c. Allow to cool and add 4 c.c. of acidified sulphite solution and bring the total volume to the 25 c.c. mark, with distilled water, if necessary. Five cubic centimeters of potassium iodide solution are added and the whole centrifuged. The clear supernatant fluid is estimated for its bismuth content as soon as possible, using the permanent standards. In case less than 4 c.c. of concentrated sulphuric acid remain in the flask after digestion, more should be added; if there is more, it should be boiled down to approximately 4 c.c. If for any reason nitric acid drips back into the flask while being cooled, yellow to brownish discoloration of the digest from liberation of iodine may occur, but this is a rare occurrence; additional boiling discharges the color, in the event of such an occurrence.

*Feces and Tissues.*—Normal animal tissues are reported to contain from 0.002 to 0.005 mg. of bismuth in 1 gm. material, or at least give a color equivalent to this amount of bismuth. We have found the equivalent of 0.0009 mg. Bi in amounts of "blank" tissue up to 25 gm., plus the necessary reagents and treatment, and always subtract this quantity from a tissue analysis. The following procedure is used with blood, liver, muscle, kidney and feces; cerebrospinal fluid is treated like urine.

The crucible (porcelain or silica) is first carefully washed in concentrated hydrochloric acid and rinsed in distilled water (to be Bi-free). Charge the crucible with 5 gm. of feces or up to 10 gm. of tissue (liver, blood, brain, kidney, or muscle) and heat on a water-bath until dry. Keep tissues covered. Six to 8 hours of drying are sufficient. (Brain and kidneys from rats and other small animals are used in quantities of 1 or 1.5 gm.) Place the crucible and contents in an electric furnace and heat gradually from 725 to 900° C. (pyrometer). Too rapid heating causes sputtering. If a sliding asbestos tray is introduced into the furnace, several crucibles may be placed on this tray and the whole gradually admitted into the furnace, which has been preheated. Considerable time can be saved in this way. Heat for twelve to fifteen hours, or overnight. The crucibles are inspected at the end of this period and any carbon still remaining may be readily oxidized by adding approximately 0.5 gm. powdered potassium nitrate and heating for another hour. Several additions of potassium nitrate may be necessary, especially with brain and liver. When a white ash is obtained, remove the crucibles from the furnace, cool, and add 5 c.c. concentrated  $\text{H}_2\text{SO}_4$  to each, making certain that the sides are washed down well with the acid. Let stand for one hour. Transfer, by washing, the contents of each crucible into a Kjeldahl flask of 300 c.c. capacity, and add 10 c.c.  $\text{HNO}_3$  and continue wet oxidation on a hot plate, as with urine. Rarely more than a single addition of nitric acid is necessary. The addition of 5 c.c. of superoxol greatly facilitates oxidation. From here on the estimation proceeds as with urine. Occasionally heating

of rabbit feces results in considerable loss of bismuth, so with rabbit feces an excess of  $\text{KNO}_3$  is used. The addition of almost any salt, such as  $\text{Na}_2\text{CO}_3$ ,  $\text{NaCl}$ , or  $\text{KNO}_3$ , before commencing the incineration, will prevent loss of bismuth. Explanation for this is not at hand, but it may be the presence of additional flux which helps to avoid loss of Bi. With blood, liver, and kidney tissue, the hot digest commonly appears yellowish in color which is presumably due to iron, but on cooling becomes colorless and the change does not affect the estimation.

Typical examples of recoveries of bismuth added to different tissues are presented in the table, including means and ranges of colorimeter readings for reagents and tissues, the blank value for subtraction in all analyses being 0.0009 mg Bi. Quantities down to 0.001 mg Bi can be accurately estimated. Quantities of the order of 0.001 mg, such as may occur in cerebrospinal fluid, have been checked qualitatively and quantitatively with a spectrograph.

TYPICAL EXAMPLES OF RECOVERED BISMUTH ADDED TO VARIOUS BODY FLUIDS AND TISSUES\*

	COLORIMETER READINGS	QUANTITIES BISMUTH ADDED		
		MG BI	COLORIMETER READING	PER CENT RECOVERY
Reagents	Mean Range		Mean	
Reagents and $\text{FeSO}_4$ (0.5 gm)	98 (75-120)	0		0
	88	0		0
Blood	101 (87-110)	0.001	94	96
Brain	99 (85-110)	0.002	91	99
Muscle	100 (84-110)	0.005	91	99
Liver	98 (81-110)	0.010	92	98
Kidney	94 (81-110)	1.000	21†	95
Feces (rabbit)	86 (76-110)	5.000	23†	87
Feces (human)	105 (101-110)	0.010	96	94

\*The compounds used were sodium iodobismuthite ( $\text{Na}_2\text{BiI}_6$ ) and sodium bismuthate ( $\text{NaBiO}_3$ ).

†Different standard level.

### CONCLUSION

Procedures for the quantitative estimation of bismuth in body fluids and tissues are outlined.

### REFERENCE

1. Hanzlik P. J., and Melstens, H. G. Clinical Excretion of Bismuth, Bismuth Metal, Arch Dermat & Syph 22: 483, 1930.



# DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

RENAL DISORDERS, Rapid Quantitative Method for Examining the Urine in, Gibbons, H. Arch. Int. Med. 54: 758, 1934.

*Enumeration of Formed Elements.*—The patient is instructed to go without fluids after breakfast the day before the test. In the evening of the day, on retiring, he empties the bladder, discards the urine and notes the time of doing so. The next morning he passes urine into a collecting bottle, again notes the time and brings the specimen to the laboratory for examination.

The volume of the collected urine is measured in a 1,000 c.c. graduate cylinder. The urine is well mixed. A volume equal to the number of hundred cubic centimeters in the total urine (i.e. 1 per cent of the total) is placed in a 15 c.c. graduate centrifuge tube. The urine is then centrifugated until all the elements are deposited at the bottom of the tube. The greater part of the supernatant urine is now pipetted off with a capillary pipette until it is reduced to the proper volume for calculation. If the centrifugated portion is from an eleven or twelve hour specimen, the volume must be 0.5 c.c., if from a nine to eleven hour specimen, the volume must be 0.4 c.c., and if from a seven to nine hour specimen, 0.33 c.c. The sediment is then mixed with the aid of a pipette, and a drop is transferred to a ruled blood counting chamber. The average number of elements in 0.1 c.mm. (one small square) is then counted. This number is divided by 2. The resulting figure is the number of million elements excreted by the patient in twelve hours. In making the count the low power objective should be used for counting casts, and the high power for the cells. This method is applicable to all specimens of urine, and gives the same picture for all similar renal lesions.

The number of elements in a twelve hour specimen is calculated by the Addis method as follows:

$$\frac{\text{Number of elements in 1 small square} \times \text{volume of specimen}}{0.0001 \text{ c.c.}} \times \frac{12 \text{ hour volume}}{\text{Volume centrifugated}} = \text{number of elements excreted in twelve hours.}$$

In these fractions it is possible to have all except two items constant. The two variable items are: (1) the number of elements counted in one small square, and (2) the twelve hour volume. It is possible to make the quotient of the fraction  $\frac{\text{Twelve hour Volume}}{\text{Volume centrifugated}}$  constant by having the denominator (volume centrifugated) always equal to 1 per cent of the numerator (twelve hour volume). When this is done the only variable item influencing the result is the number of elements counted in one small square. Furthermore, when, instead of a twelve hour specimen the patient brings an eight, nine, or ten or eleven hour specimen, it is necessary to correct the volume to twelve hours. But the calculation for this can be avoided by taking the centrifugated sediment up in, say, eight-twelfths of 0.5 c.c., or 0.33 c.c. for an eight hour specimen; nine-twelfths or 0.37 c.c., for a nine hour specimen; ten-twelfths, or 0.41 c.c., for a ten hour specimen, or eleven-twelfths, or 0.45 c.c. for an eleven hour specimen. Because the error in the final result is small, it is unnecessary to correct closer than a two hour difference. Thus, a seven to nine hour volume is taken up in 0.33 c.c., and a nine to eleven hour volume is taken up in 0.4 c.c., and the different times can be so arranged that the number of elements counted when divided by 2 will give the result in millions of elements per twelve hour specimen.

For example 1 Given a twelve hour specimen of urine, the volume is 400 cc and 12 red blood cells are counted in one of the nine squares of the counting chamber. Therefore, according to the formula

$$\frac{12 \times 0.5 \text{ cc} \times 400 \text{ cc}}{0.0001 \text{ cc} \times 4 \text{ cc}} = 6,000,000 \text{ red blood cells}$$

2 Given a ten hour specimen the volume is 333 cc, with the precipitate taken up in 0.4 cc (note that the volume does not change in the calculation) and 12 red blood cells are counted in one of the nine squares

Therefore

$$\frac{12 \times 0.5 \times 333 \text{ cc}}{0.0001 \times 3.3} = 1,000,000 \text{ red blood cells}$$

*Estimation of Urine Protein*—In order to know the rate of output of albumin, it is necessary to know the quantity excreted over a definite period of time. Added calculated the excretion of protein of normal and diseased kidneys for twelve hour periods. By simple mathematics the volume of urine excreted during twelve hours can be estimated from the volumes collected in from eight to eleven hours. Then, with 1 cc of urine, a calculation of the percentage of protein present is made and from this information the excretion of protein in twelve hours is determined. For this estimation the simple and rapid procedure for measuring albumin described by Lashmet and Newburgh has been adapted. The turbidity, or cloud, produced by adding sulphosalicylic acid to the urine containing protein is compared with that of a standard turbidity solution representing a known protein content.

*Stock Solution*—To about 200 cc of distilled water in a 500 cc volumetric flask, 50 cc of a tenth normal solution of sodium hydroxide USP, and 8 gm of cupric sulphate, USP (hydrous) are added. The volume of 500 cc is made with distilled water.

*Standard Solution*—After shaking the stock solution vigorously to insure uniform suspension, exactly 4 cc of this mixture is transferred to a 100 cc graduate cylinder and tap water is added up to the 50 cc mark. The turbidity of this mixture is the same as that produced by a 0.1 per cent solution of protein. 0.1 gm per one hundred cubic centimeters, when sulphosalicylic acid is used. This solution can be kept in a flask for use when wanted. The turbidity does not change on standing. It must however, be shaken several times before using.

In order to determine the amount of protein in the urine 1 cc of the centrifugated urine is placed in a 100 cc graduate cylinder and a 2 per cent solution of sulphosalicylic acid is added to bring the volume up to 25 cc. The standard solution is placed in a second 100 cc cylinder for comparison. If the turbidity of the mixture is the same as that of the standard solution the urine contains 0.1 per cent protein. If the turbidity is greater than that of the standard solution the mixture should be diluted with tap water until it compares with the standard. Correction for the dilution is done by multiplying 0.1 per cent by the number of times the 25 cc volume has been diluted, then the corrected figure is multiplied by the number of cubic centimeters of urine excreted in twelve hours. The result is the number of grams of protein excreted in twelve hours. If the turbidity of the mixture is less than that of the standard solution, urine should be added until the same turbidity as that of the standard solution is obtained. The twelve hour volume is then divided by the number of cubic centimeters of urine added and multiplied by 0.1 per cent. Not more than 5 cc of urine should be added to the reagent, if no cloud is formed with this amount, the albumin content of the urine is negligible. This is a rough estimate of the rate of excretion of protein, and is applicable for practical use in determining the type or the progress of a renal lesion.

**WHOOPIING COUGH, Blood Picture in Experimental,** Inaba I and Inamori S. Am J Dis Child 48 1193, 1934

The authors succeeded in producing experimental whooping cough by means of infection in eight young monkeys and six puppies. Except in one mild case, leucocytosis and lymphocytosis were observed. The symptom appeared after from three to four weeks of

incubation, often before the development of paroxysmal cough, and therefore is not a mechanical effect of cough but a biologic reaction caused by infection with the pathogenic bacillus. After the infection and before the appearance of lymphocytosis, no leucopenia could be proved. For the diagnosis of whooping cough and the determination of its stage, it is much more profitable to depend on the absolute number of lymphocytes than on the total leucocyte count and the percentage of lymphocytes, especially when complications are present.

**SPINAL FLUID, Diagnosis in Spinal Fluid Contaminated by Blood: The "Bloody Tap,"**  
Soloman, P. New England J. M. 212: 55, 1935.

The following criteria are useful:

	"Bloody Tap"	Previous Subarachnoid Bleeding
Homogenous admixture of blood	0	+
Clot formation	+	0
Xanthochromia in supernatant fluid	0	+

The following proportion enables a leucocyte count in bloody fluid:

R.B.C. b = peripheral R.B.C. count

W.B.C. b = peripheral W.B.C. count

R.B.C. f = fluid red cell count

W.B.C. f = fluid leucocyte count

W = leucocytes originally in fluid

$$\text{Then: } W = W.B.C. f - \frac{W.B.C. b}{R.B.C. b} \times R.B.C. f$$

Example: Leucocyte count on peripheral blood = 20,000

W.B.C. count in spinal fluid = 260

R.B.C. count in spinal fluid = 25,000

$$\text{Then: } W = 260 - \frac{20,000}{5,000,000} \times 25,000 = 260 - 100 = 160$$

Calculation of Protein Content: P

P = original protein content of fluid

R.B.C. b = red cell count of patient

Pb = serum protein

H = hematocrit value of blood

Pf = protein content of supernatant fluid

For most purposes Pb can be taken as 7 gm. per cent; H as 43 per cent; R.B.C. b as 5,100,000

$$\text{Then } P = Pf - \frac{R.B.C. f}{R.B.C. b} \times Pb \times (1-H)$$

or, with the average values given above:  $P = Pf - 0.0008 \text{ R.B.C. } f$ .

The following dicta represent good approximation.

1. To obtain the original white cell count in the spinal fluid, subtract 1 leucocyte for every 500 red cells.

2. To obtain the original protein content subtract 4 mg. per 100 c.c. for every 5,000 red cells (or, roughly, 1 mg. per 1,000 red cells).

3. Sugar and chloride values are not appreciably affected by contaminating blood.

4. The colloidal gold reaction is not affected unless the red cells are over 5,000. Even 25,000 produce only a few 2's in the curve.

5. A positive reaction in bloody spinal fluid is significant only when the blood Wasserman is negative.

**POLIOMYELITIS, A Successful Method for Vaccination Against,** Kolmer, J. A., Klugh, G. F., and Rule, A. M. J. A. M. A. 104: 456, 1935.

Twenty-five children varying in age from eight months to fifteen years have been given from one to three injections of poliomyelitis vaccine at the request or with the consent of parents.

Fifteen of these children were without antibody in serum neutralization tests before immunization and eleven or 75 per cent showed large amounts of antibody in the blood one week after the last dose of vaccine.

Ten of the children showed the presence of antiviral antibody in the blood before immunization, but all have shown a considerable increase of this antibody after vaccination, so that antibody production occurred in twenty one or 84 per cent of the group of twenty five children.

None of the twenty five children have shown the slightest ill effects from the vaccine.

Mild local reactions were produced at the sites of subcutaneous injection with occasional slight elevation of temperature and slight leucocytosis subsiding within forty eight hours.

The dosage for children from one to three years of age has been 0.25, 0.5, and 0.5 c.c. at weekly intervals, for children from four to ten years, 0.5, 0.5, and 1.0 c.c., for children from eleven to fifteen years 0.5, 1 and 1 or 2 c.c. For adults the dosage recommended is 0.5, 1, and 2 c.c.

The vaccine is prepared of spinal cord only, as brain contains too small amounts of virus. But the spinal cord of a single monkey will furnish about 150 c.c. of vaccine which is sufficient for the immunization of from forty to seventy five children, depending on age and dosage.

It is likely that the maximum antibody response may be obtained by giving the injection every ten days instead of weekly.

Antibody production, however, appears to be fairly rapid, as three susceptible children developed antibody in the blood within four days after the first injection of vaccine and one monkey was found completely and a second partially immune seventy two hours after the subcutaneous injection of 0.5 c.c. of vaccine per animal on intracerebral inoculations of large amounts of virus.

The vaccine does not appear to produce a demonstrable "negative phase" of increased susceptibility after injection.

The vaccine is a 4 per cent suspension of spinal cords of monkeys developing poliomyelitis after intracerebral inoculation with a remote passage strain of virus, in a 1 per cent sterile solution of sodium ricinoleate prepared as previously described. The virus is attenuated and the vaccine regarded as entirely safe for the immunization of human beings not only because prepared of remote passage virus which probably has lost its infectivity for human beings but likewise because of attenuation by sodium ricinoleate the route of administration and the injection of a small first dose.

The amount of antibody produced by immunization is comparable to that found in the blood in natural immunity and is believed to be sufficient for affording protection against acute anterior poliomyelitis.

The antibody present in the serums of vaccinated children has successfully neutralized human virus from the 1934 California epidemic.

The duration of the immunity following vaccination is unknown but has lasted for more than three years in vaccinated monkeys.

It is believed that the vaccine is now ready for vaccination of human beings and especially children against poliomyelitis and particularly during epidemics.

#### CARCINOMA, Early Cutaneous, Sutton R. L. J. A. M. A. 104: 437, 1935

Sutton formulates the following postulates:

1 (a) Many skin cancers begin as *de novo* lesions. (b) The earliest visible lesion in these cases is a circumscribed scaly, epithelial newgrowth.

2 (a) The structure of many minute scaly epithelial newgrowths is such that it is reasonable to presume that if not interrupted they would become obvious carcinomas. (b) It is reasonable to believe that such lesions are in fact early carcinomas. (c) If a lesion has a structure not compatible with a likelihood of its being early carcinoma it might be called precancerous. But it would be impossible to predict that such a lesion might develop a structure, if uninterrupted, such that it would be properly called carcinoma.

3. (a) It is impossible to determine at what point in its natural history a cancerous lesion was not cancerous. (b) It is reasonable to believe that cancer is cancer from the start.

4. The concept of precancerosis is indecisive and undefinable. It groups unrelated conditions, which may or may not be early cancer. Its acceptance entails an insoluble problem of a dividing line between cancer and noncancer, as well as an insoluble problem of statistical assay of lesions that are strictly individual.

5. (a) A lesion may be cancerous independently of its size and rate of growth. (b) Cancer is primarily an epithelial disease. (c) A cancer consists of mutated somatic cells. (d) The earliest visible manifestations are circumscribed, dyskeratotic lesions which microscopically are composed of polymorphous epithelial cells that proliferate, keratinize and undergo mitosis in an abnormal manner. (e) Malignancy depends on a balance between the proliferative capacities of its cells and the control or resistance of the host. (f) One tumor may contain several kinds of cells as a result of mutation following on mutation.

6. Early cancerous lesions are readily destroyed and cured. In suspecting all early lesions and destroying them, one would prevent the development of all late lesions such as might become incurable.

**RENAL FUNCTION: Sodium Ferrocyanide as a Clinical Test for Glomerular Efficiency,**  
Stieglitz, E. J., and Knight, A. A. J. A. M. A. 103: 1760, 1934.

The method follows: 0.5 gm. of hydrated salt, or about 0.25 gm. of the anhydrous salt is prepared in sterile ampules (those for the study were supplied by the Abbott Laboratories).

The contents of one ampule completely dissolved in water is intravenously injected and urine specimens collected at 30, 60, 120 and 180 minutes after the injection.

The Titration: The titration is carried out with a quantitative copper sulphate solution made to contain 0.4 per cent of copper sulphate (0.004 gm. per cubic centimeter). The content of copper is determined exactly by electrolysis. Before titration the specimens are acidified with concentrated sulphuric acid. Copper ferrocyanide is an insoluble red salt but is less insoluble than the well-known Prussian blue. This fact is used in determining the end-point of the titration. Drops of concentrated ferric chloride solution are placed on a tile and as the titration proceeds a drop of the unknown solution is placed in contact with the ferric chloride. If free ferrocyanide is present an immediate formation of Prussian blue occurs; if all the ferrocyanide has been precipitated as cupric ferrocyanide, a distinct delay occurs in the appearance of this blue color on the tile. The more insoluble ferri-ferrocyanide slowly replaces the slightly more soluble cupric ferrocyanide. The end-point therefore is read when the appearance of blue is delayed appreciably (five seconds), and the number of cubic centimeters of copper sulphate required is noted.

If hematuria exists during the period of ferrocyanide elimination the method of titration must be modified, for the sulphuric acid apparently liberates sufficient ferric ions from the hemoglobin to precipitate Prussian blue and thus prevent the formation of the red cupric ferrocyanide. This difficulty is readily avoided by determining the volume of the specimen of urine, bringing it to a quick boil, which precipitates the protein, and then filtering it. A measured quantity of this filtrate is then acidified with concentrated sulphuric acid and titrated in the usual way, the results being corrected to the original volume of the specimen.

The following conclusions were reached:

Sodium ferrocyanide, in doses of 0.5 gm. of the hydrated salt (0.25 gm. of anhydrous sodium ferrocyanide) has been used in more than 100 individuals for the purpose of studying the glomerular efficiency.

It has proved entirely nontoxic in this dosage, when slowly administered intravenously.

The normal secretion curve is characteristic and shows but relatively little spread of variation. This variation is less than that of phenolsulphonphthalein excretion.

So far as is known, ferrocyanide salts are excreted solely by way of the glomeruli.

In hypertensive arterial disease the excretion of ferrocyanide is considerably retarded, much more so than the phenolsulphonphthalein elimination.

In congestive heart failure both the phenolsulphonphthalein and the ferrocyanide elimination are impaired.

In known glomerulonephritis the excretion of sodium ferrocyanide is either nil in severe cases or else very much reduced.

This test offers notable potentialities of clinical usefulness, since it is specific for the glomeruli, is safe, is simple of execution, and is quite constant in the normal.

In physiologic investigations of the mechanism of renal secretion the procedure should also prove of considerable value.

Much further work is required before the full significance and import of these studies are understood. The authors hope that this preliminary report may encourage others to extend and amplify this work, it has been their object merely to present a new method and to point out some of its potentialities.

#### ANEMIA of Prematurity, Josephs, H. W. *Am J Dis Child* 48 1237, 1934

A group of premature babies has been studied during the first three or four months of life, for the purpose of determining, if possible, the mechanism of the physiologic anemia of prematurity. Importance has been attached to the response of the reticulocytes and especially its relation to the administration of iron. From the study of the reticulocytes it was concluded that there is no basis for the idea that the fall in red cells and hemoglobin was dependent on hypoplasia in any strict anatomic sense. There was a period of failure to react to the administration of iron that lasted for from six to ten weeks after birth, the duration of the period depending largely on the degree of prematurity. After this early period of nonreactivity there followed a short transition period with delayed response, after which administration of iron was followed by a prompt response of the reticulocytes and a rise in red cells and hemoglobin. There is no reason to believe that lack of stores of iron plays any part in the development or persistence of the anemia before the end of the third month. Thereafter, the rise in red cells without a corresponding rise in hemoglobin in untreated infants indicates the possibility that lack of stores of iron has become an important factor. From the results reported here one cannot conclude that a low percentage of hemoglobin is in itself harmful although it probably is indicative of a condition that renders the infant more likely to succumb to infection. In such cases, although iron may raise the hemoglobin content, transfusion must still remain the method of choice in treating the condition as a whole. The evidence from this study does not permit the conclusion that liver is necessary as an adjunct to iron, though in individual cases it may prove of benefit. As in the case of iron, liver given alone in the early period was ineffective, but given later was followed by a response which differed in no way from that following the administration of iron. Copper had no demonstrable effect.

## REVIEWS

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Books and Monographs for Review should be sent direct to the Editor,  
Dr Warren T. Vaughan, Professional Building, Richmond, Va

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### Laboratory Diagnosis\*

THOSE familiar with the first edition of Osgood and Haskin's book on Laboratory Diagnosis will not be surprised to find that a second edition of this useful and practical text has become necessary.

The present volume, dedicated to the memory of the coauthor of the first edition, has been extensively and thoroughly revised by Dr Osgood and embodies much new material representing the newer advances in the field of laboratory medicine.

Among the more important changes are discussions of the blood urea clearance, the insulin coefficient, the determination of blood bromides, the Friedman test, the galactose tolerance test, the heterophile antibody reaction, medicolegal applications of blood grouping, and the quantitative determination of proteins in spinal and other body fluids.

Widely known for his studies in hematology, Dr. Osgood<sup>1</sup> presents the results of many investigations relating to hematologic technique as well as concerned with normal values.

Despite the extensive changes throughout the text generally, the original plan of the book has not been altered.

Clinical pathologists have long decried the misuse of laboratory procedures which, after all, if they are to fulfill their true function, must be regarded, not as diagnostic tests, but as phases in the examination of the patient requiring not only intelligent application but, particularly, intelligent interpretation.

The first section of this book, therefore, is devoted to a discussion of the application of laboratory procedures in the study of disease and suggests the answers to the crucial questions. What are the laboratory studies most likely to be informative in a particular problem? When and how often should they be utilized? What is the clinical significance of their results?

The second part of the book is devoted to technical methods.

The style throughout is clear, simple, and without ambiguity. The illustrations are equally good.

A feature of the book is the double index. An index by diseases enables the practitioner to determine in a particular condition what laboratory methods are of material aid and refers him to a discussion of their clinical significance and interpretation.

A general index makes the contents of the book readily available.

It may be said with confidence that this book constitutes a practical, comprehensive, and authoritative text destined to become a standard reference.

It may be recommended without reserve to student, practitioner, and laboratory worker alike.

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### Failure of the Circulation†

THIS is a book for which the physician at large has been waiting, whether he knows it or not. Certainly it presents in a clear, interesting, and comprehensive fashion, a well conceived and equally well carried out concept of the hemodynamic aspects of cardiac and circulatory disease as considered from both the clinical and physiologic aspects.

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\*A Textbook of Laboratory Diagnosis. By Edwin E. Osgood, M.D., Assistant Professor of Medicine and Biochemistry, University of Oregon School of Medicine, Cloth, pp 385, 27 figures, 10 colored plates. P. Blakiston's Son & Co., Philadelphia, Pa.

†Failure of the Circulation. By Tinsley R. Harrison, M.D., Associate Professor of Medicine, Vanderbilt University School of Medicine. Cloth, pp 396, 60 figures. Williams & Wilkins Co., Baltimore, Md.

The book is divided into five main sections. I The Hypokinetic Syndrome, in which the clinical picture as well as the underlying causes of cardiac insufficiency are considered, II The Hyperkinetic Syndrome, which discusses the overactive heart and cardiac neurosis, III The Dyskinetic Syndrome in which the inefficient cardio-circulatory system is discussed under these subdivisions: A General Clinical Considerations, B Dynamics of Congestive Heart Failure, C The Major Phenomena of Congestive Heart Failure, D The Minor Phenomena of Congestive Heart Failure, E The Prognosis and Treatment of Congestive Heart Failure.

Part IV discusses Mixed Types of Circulatory Failure and Part V Failure of the Coronary Circulation.

In the last analysis, there are but four main problems confronting the physician in the consideration of the cardiovascular system of a patient. These, as stated by Maher are (1) What caused the cardiac disease? (2) What is the structural lesion? (3) How is the physiology changed? and (4) What are the patient's limitations?

In their elucidation Dr. Harrison's book will be of great value and assistance. It may be recommended without reserve as an excellent and valuable contribution.

### The Harvey Lectures\*

IN THIS volume, the twenty-ninth series of *The Harvey Lectures*, the following topics are authoritatively discussed: Typhus and Rocky Mountain Spotted Fever in the United States, by Dr. R. E. Dyer, The Potential Energies of Oxidation-Reduction Systems and Their Biochemical Significance, by Dr. W. Mansfield Clark, Hereditary Grafting in Embryology, by Dr. Ross G. Harrison, The Estrogenic Substances, by Dr. E. A. Doisy, The Clinical Application of Some Recent Knowledge of the Biliary Tract and of the Pancreas, by Dr. Everts A. Graham, The Significance of Morbid Processes in the Fetus, by Dr. George L. Streeter, Filterable Viruses With Special Reference to Parotitis, by Dr. Thomas M. Rivers, and The Nervous Mechanism of Cardiovascular Control, by Dr. David W. Bronk.

As always these volumes constitute a welcome and valuable addition to the physician's library.

### Quarterly Bulletin of The Health Organization, League of Nations†

THE Special Number issued by The Health Organization of the League of Nations comprises the report of the Permanent Commission on Biological Standardization on proposed international standards for gas gangrene antitoxin, anti-neumococcus serum, Types I and II, and Staphylococcus Antitoxin.

The issue of December 1934 contains a lengthy report of the Demographic Setting of Infant Mortality, The Fifth Analytical Review of Reports from Pasteur Institutes on the Results of Anti-Rabies Treatment, A Brief Guide to the Varieties of *Anopheles Maculopennis*, A Report on Milk Hygiene in the Department of Meurthe et Moselle, and Current Notes on the Work of The Health Organization.

### A Textbook of Histology‡

IN THIS volume Cowdry presents histology in not only an interesting and vivid fashion but also in such a manner that it can be applied to the problems of everyday medical practice.

As he remarks in the Preface, histology cannot be regarded as in the past it has been to some extent, as an isolated subject but must be accepted as an integral part of the study

\*The Harvey Lectures. Delivered Under the Auspices of The Harvey Society of New York under the patronage of the New York Academy of Medicine. Cloth pp. 262. Williams & Wilkins Co. Baltimore, Md.

†Quarterly Bulletin of The Health Organization, League of Nations. III: 1 December 1934 and Special Number January 1935.

‡A Textbook of Histology. Functional Significance of Cells and Inter-cellular Substances. By L. V. Cowdry, Professor of Cytology, School of Medicine, Washington University, St. Louis. Cloth 11 503 242 engraving. Lea and Febiger Philadelphia, Pa.



of the body, and as having an interlocking relationship with normal function, physiology, on the one hand, and abnormal function, pathology, on the other.

In considering the properties and functions of cells it must be recognized that there is no such thing as a generalized kind of cell existing alone. All are influenced by their heredity, the fluid about them, and the other cells with which they are associated.

The aim of this volume, therefore, is to build up a conception of cells and primary tissues in the many environments in which they normally exist and to stress the organs and systems in such a manner as to relate structure and function in the whole body, which is itself a physiologic unit.

The principal sections into which the book is divided are: Water, The Essential Vital Medium, The Blood, The Principal Integrator, Absorptive Drainage into the Blood, Chemical Integration by Endocrine Products in the Blood Stream, Intake of Water, Nutriment, Accessory Food Factors, and the Removal of Waste, Oxygen Consumption and Carbon Dioxide Elimination, Rapid Neural Integration, Architectural Structure, Perpetration of the Race, Unification, Protection, and Adjustment.

The practical conception of histology and its excellent presentation in the text result in a volume of interest and use to student, practitioner, and pathologist alike to whom it may be recommended without reserve.

The format and illustrations are in keeping with the volumes of this publisher.

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### The Spleen and Resistance\*

THAT the mechanisms governing the development of natural resistance are of great and obvious importance has long been recognized and that the spleen is in some way concerned with these phenomena has long been known. The exact nature of the function of this "enigmatic organ," as Marine well calls it, yet remains to be elucidated but the researches presented in this volume constitute a definite advance.

Beginning with a brief chapter on anatomic considerations the authors then consider in some detail the pathological changes in the spleen in a variety of infections, acute, chronic and virus diseases as well; the spleen as an organ of macrophage tissue; its relation to antibody formation; the effect of splenectomy on natural resistance; the relation of the spleen to acquired resistance in latent infections; compensatory changes following splenectomy; the significance of depression in resistance following splenectomy; and, finally, the variability of the effect of splenectomy in animals and its significance in the interpretation of the physiology of the spleen.

The survey thus presented embodies not only a comprehensive and critical review of the literature but also the results of the experimental investigations of the authors and, in all, constitutes, perhaps, the first systematic presentation on the subject.

A final résumé together with a bibliography and subject index enhance the value of the book.

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### The Story of Medicine in the Middle Ages†

THAT interest in the history of medicine has not been as acute and as general among physicians as it should be, may have been accounted for by the relative dearth, until recent years, of volumes such as this. For it will be received enthusiastically not only by those now interested in medical history but without doubt will arouse equal interest in those who may have been dismayed by more ponderous tomes.

Dr. Riesman has chosen for his subject the history of medicine during the period commonly spoken of as "the Dark Ages" for, curiously enough, as he says in his Preface, there is more familiarity with the mythology of Greece and the history of ancient Rome than with the period called "the Middle Ages" although this period is so much nearer to us.

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\*The Spleen and Resistance. By David Perla, M.D., Associate Pathologist, Montefiore Hospital, and Jessie Marmarston, M.D., Associate in Pathology, Cornell Medical School, With a Foreword by David Marine, M.D. Cloth, pp. 170. The Williams & Wilkins Co., Baltimore, Md.

†The Story of Medicine in the Middle Ages. By David Riesman, M.D., Professor of the History of Medicine, University of Pennsylvania. Cloth, pp. 402, 79 figures. Paul B. Hoeber, New York.

The account which he gives of the development of medical lore and practice and of those associated with it is not only authentic and comprehensive, but written with charm and sympathetic understanding. It is a easily flowing story filled with incident, light, color, and vivid pictures in which the actors appear not as shadowy phantoms dimly seen through the murky mists of long dead centuries but as living worthies sharply etched against the panorama of their times.

It is a book which one suspects gave the author as much enjoyment in the writing as the reader will derive from its perusal.

Here are tales not only of individual struggles and achievement, but of the times in which they occurred, of astrology and astrologers, alchemy and alchemists, the surgeons and barbers of Paris, medieval baths and sanitation, medieval epidemics, leprosy, the sweating sickness and St. Anthony's Fire, the King's evil and epilepsy—all of which left their mark upon medicine and its development and here they are seen as vivid actualities.

Both author and publisher are to be congratulated upon a worth while book, one which can be read and reread and the reader perhaps, is to be congratulated most of all that it has been made available.

### International Medical Annual\*

NOW in its fifty third consecutive year, the *International Medical Annual* needs no introduction for it has been the desk companion of innumerable physicians ever since the first volume appeared.

As before, the book begins with a review of the year's work in the treatment of disease followed by the characteristic terse yet ample summaries of the literature on disease and its treatment. As usual the subjects are alphabetically arranged, which with the comprehensive index makes the subject matter readily accessible.

As an authoritative reference to recent advances the volume can be recommended with out reserve.

### Laboratory Manual†

THIS small volume, prepared for the guidance of students and staff members, presents the routine methods in use in Peiping Union Medical College.

Intended as a guide to laboratory methods the text is restricted entirely to technique. Despite its size the book is quite comprehensive. Directions are detailed and clearly given and the book may be accepted as a safe guide for the methods described.

### The Kidney in Health and Disease‡

THIS volume is the outcome of a symposium on the structure and function of the kidney in health and disease conducted at the University of Minnesota a few years ago under the direction of Dr. Hilding Berglund, then Professor of Medicine in that institution.

The contributions then made amplified and revised to cover recent advances, constitute the material now presented and represent probably the most comprehensive survey of this subject yet published under one cover.

Each of the forty one contributors is an accepted authority in the field whereof he writes.

\*International Medical Annual. Edited by H. Lethbridge Tilly and A. Renlie Short. Cloth pp 529 64 plates. William Wood & Co. Baltimore Md.

†Laboratory Manual of the Department of Bacteriology and Immunology of Peiping Union Medical College. By C. E. Lim. Ed 2. Cloth pp 190. Kwang Yung Press. Peiping China.

‡The Kidney in Health and Disease. Edited by Hilding Berglund M.D. formerly Chief Department of Medicine University of Minnesota and Grace Medes Ph.D. Research Biochemist Lankenau Hospital Research Institute. Cloth pp 774 163 figures. Lea and Febiger Philadelphia Pa.

The volume is divided into six main sections: I. Anatomy and Physiology; II. Clinical Aspects of Renal Functions; III. Bright's Disease and Other Pathologic Renal Conditions; IV. Albuminuria and Edema; V. Ocular Changes in Bright's Disease; and VI. Clinical Aspects of Bright's Disease.

As these headings indicate, the book is not a presentation of abstract theories of interest only to the research worker, but is replete with material of real interest and practical value to the practitioner, the pathologist and, indeed, to all concerned with the kidney in its relation to disease.

The sections on the Clinical Aspects of Renal Function, Bright's Disease, Albuminuria and Edema, and the Clinical Aspects of Bright's Disease will be of great interest and value to the practitioner.

It is obvious that the last word upon the functions and diseases of the kidney has not yet been written and that there are many aspects of this subject which must still be the subject of controversial discussion. Nevertheless the present contribution furnishes a comprehensive and well planned survey of the status quo at present.

There is, naturally, some slight unevenness in the assembling of such varied material from numerous contributors but the editors deserve much credit for the care with which the material has been arranged and correlated.

The format and the illustrations are excellent.

An author's index and general index make the material readily accessible.

This book deserves a place in the physician's library.

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### The Woman Asks the Doctor

THIS little book is addressed to the laity, especially to women who, as the author remarks in his Preface, "are eager to know something of the significance of the remarkable cyclical phenomena which characterize their sex, particularly as these have for centuries been enshrouded in a mantle of mysticism."

The value of accurate information on this subject is apparent and yet, unfortunately, it is all too often disseminated by dubious routes and is often garbled and misleading.

The doctor and the gynecologist, who should be the proper avenues for instruction in these matters, should welcome Dr. Novak's book as covering the subject fully and yet simply.

The scope of the volume is apparent from the chapter headings: What Is Femaleness; Superstition and Folklore of Menstruation; The Reproductive Apparatus of Women; The Cause and Significance of Menstruation; The Glands as Related to Female Functions; The Beginning of Womanhood (Puberty), Characteristics of Normal Menstruation; The "Change of Life" (Menopause); Hygiene of Menstruation; Woman as an Egg Producer; With Remarks on the So Called "Safe Period"; Some Common Disorders of Menstruation; Sterility in Women; Leucorrhea; Cancer, The Arch Enemy of Women; A Little About the Sex Life of Women.

This book may be safely recommended to the audience to whom it is addressed.

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### Annals of the Pickett-Thomson Laboratory†

IN this volume the authors present a comprehensive survey concerned with the literature of influenza with special reference to the complications and sequelae, bacteriology of influenzal pneumonia, pathology, epidemiological data, prevention, and treatment.

The thoroughness with which the task has been carried out is exemplified by the bibliography embracing 4,500 papers.

Those fortunate enough to have seen the previous volumes published by these workers will find the present one of equal interest and value.

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\*The Woman Asks the Doctor. By Emil Novak, Associate in Gynecology, Johns Hopkins Medical School. Cloth, pp 189, 11 figures. The Williams and Wilkins Co., Baltimore, Md.

†Annals of the Pickett-Thomson Research Laboratory. Vol. X (Part II), Influenza. By D and R Thomson. Paper, pp 157. The Williams and Wilkins Co., Baltimore, Md.

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## EDITORIAL

### Tissue Penetration

THERE has recently been described a curious property of certain substances in promoting the spread of themselves or of other substances from a central focus into the tissues. This applies to various tissues but most especially to the subcutaneous tissues. The most recent work on this line has evolved from the observation of Duran Reynals that testicle extract had the power of diffusing through tissues.

Claude has continued these investigations and has found that a diazo compound of sulphanilic acid possesses the same diffusing property. Probably the most interesting part of this observation, however, is that it not only diffuses itself but also other particulate matter. Thus, if it is given intracutaneously into the skin of a rabbit along with India ink, the India ink diffuses out through the tissues many times farther than when India ink is given in the same way but with physiologic saline instead. Diazo compounds of anilin and of arsanic acid reacted likewise.

Claude next carried his investigation into the diazotizing of the aromatic amino acids, tyrosine, and histidine.

The simpler compounds had been quite irritating to the skin but these newer compounds were nonirritating and produced even greater spread. He finally diazotized protein and found that these azoproteins penetrated the skin and carried the India ink to even greater distances. These also were nonirritating.

Apparently any protein could be coupled with the diazo compound with the resulting azoprotein exhibiting this penetrating ability. The proteins used were serum, egg albumen, and gelatin. The degree of penetration varied and appeared to vary depending upon the content of tyrosine and histidine in the protein. The diazotizing process appeared to require tyrosine or histidine or some other eyelie grouping. The activity of the azoprotein in tissue penetration appears to be dependent upon its content of these partieuclar amino acids. The spreading power of azoprotein appears to depend on the number of diazo groups attached to the protein molecule.

A normal spread of the India ink indicator covers an area of about 6.8 sq. cm. whether the additional solution be Ringer's solution, normal horse serum, or sulphanilie acid. When the last of these has been diazotized, the spread averaged 22.3 sq. cm. The spread with azogelatin was 31.7 sq. cm. with azoserum about 70, and with azoalbumin 137 sq. cm.

This ability of azotized protein to penetrate long distances through the subcutaneous tissues and to carry other matter with them holds promise of being a very interesting phenomenon, possibly with some potentialities for practical application. The simpler compounds, particularly azotized analin products are highly bactericidal. This suggests the possibility of the introduction and distribution of a germicide in subcutaneous infection. On the contrary we must recall as stated above, that testicular extract appeared to enhance bacterial infection, probably by this same method of promoting spread. Claude remarked "So far there is no evidence that azoproteins and testicle extract have a common mechanism of action, although their ultimate effect on tissue permeability may be the same."

Unfortunately the higher compounds, especially the azoproteins have lost completely their bactericidal effect in vitro. But, if such compounds will carry India ink with them they might also carry germicidal substances in solution, provided such a substance could be developed which would be highly toxic for bacteria and relatively nontoxic for tissues.

One might also conceive of the possibility of the development of a method of therapeutic penetration of tumors.

At present none of these possibilities are in the offing and attention is called to Claude's observation merely as a curious and interesting phenomenon which merits further study.

#### REFERENCE

Claude, Albert: Spreading Property of Azoproteins in the Dermis, J. Exper. Med. 62: 229, 1935.

W. T. V.

# The Journal of Laboratory and Clinical Medicine

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## CLINICAL AND EXPERIMENTAL

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### BACTERIOLOGIC STUDIES ON THE FECAL STREPTOCOCCI AND THE LACTIC ACID STREPTOCOCCI\*

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A L KLECKNER M S, PHILADELPHIA, PA

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MANY workers seem to be of the opinion that there is a great deal of overlapping in the classification of certain species of bacteria. A number of species are described and named which, from their general reactions, apparently do not differ greatly from other species classified in the same genus.

With this in mind, and the fact that numerous references in the literature have stated that *Streptococcus fecalis* and *Streptococcus lactis* are or seem to be identical, this work was begun. The purpose was to study a number of organisms of each species simultaneously, subject them to the same conditions of growth, and carry out various tests in order to note any differences in their reactions which would warrant classification as separate and distinct species.

Some of the earlier workers who noticed the similarity between *Streptococcus fecalis* and *Streptococcus lactis* were Kruse,<sup>1</sup> Sittler,<sup>2</sup> and Schmitz.<sup>3</sup> Later, in 1924, S. H. Ajers and W. T. Johnson, Jr.,<sup>4</sup> became interested in this matter when they noted a similarity of reactions between the fecal streptococci and their cultures of *Streptococcus lactis*. They formulated the conclusion that *Streptococcus fecalis* or enterococcus, "is similar to, if not identical with, *Streptococcus lactis*." They further claimed that the question still remained unanswered but more clearly understood.

Schmitz,<sup>5</sup> Meyer and Schonfeld,<sup>6</sup> Calleno,<sup>7</sup> and Gandel,<sup>8</sup> each working on either fecal or lactic acid streptococci, state that it is their belief that these two organisms are identical. Karl J. Detmeyer, in 1929,<sup>10</sup> stated that "there is no

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\*From the Laboratory of Hygiene School of Medicine University of Pennsylvania  
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real difference between *Streptococcus lactis* and *Streptococcus fecalis* and that the name *Streptococcus lactis* may be used to define a local form of *Streptococcus fecalis*, more or less adapted to milk medium."

Grossman<sup>11</sup> states that the question of whether *Streptococcus lactis* and *Streptococcus fecalis* are identical needs further investigation.

There are numerous references in the literature pertaining to the use of the term *Streptococcus fecalis* or enterococcus. Thierelein<sup>12</sup> found a gram-positive diplococcus in the intestine, in normal stools, and in purulent discharges, which he named enterococcus. Although the description which he gave the organism does not agree with later descriptions of an organism given the same name, the later description is usually the one considered when using the term enterococcus, i.e., large, oval-shaped cocci, appearing in pairs or short chains, capable of fermenting "sugars," usually heat resistant, and generally not pathogenic for laboratory animals.

Andrews and Horder<sup>13</sup> gave the name *Streptococcus fecalis* to an organism found in great numbers in the intestinal tract. Dible,<sup>14</sup> who has made an extensive study of the enterococci, claims that *Streptococcus fecalis* of Andrews and Horder is of the same group as the enterococcus, while *Streptococcus enteritis* (Libman) is a true chain-forming coccus of the type found in the oral cavity, and may be a surviving form of this organism. The terms *Streptococcus fecalis* and enterococcus are used hand-in-hand in this paper to denote the same organism.

The major cultural characteristics of members of the *Streptococcus fecalis* and *Streptococcus lactis* groups are given in Tables I and II, which were made up from the summaries of the various papers by the authors mentioned.

#### SOURCE OF CULTURES AND ISOLATION

The cultures for this study were isolated directly from feces and milk or milk products, such as buttermilk and cottage cheese.

*Isolation.*—*Lactic Acid Streptococci:* Numerous samples of unpasteurized milk, buttermilk, and cottage cheese were obtained. The buttermilk and cheese samples were plated out immediately upon receiving, in dilutions of 1:1,000 and 1:10,000 on bromthymol blue dextrose agar. Part of each sample was also streaked directly on bromthymol blue dextrose agar and incubated at 30° C. until colonies of streptococci were visible. The milk samples were allowed to stand at room temperature until the acidity reached approximately 0.2 per cent. They were then plated out on bromthymol blue dextrose agar in a dilution of 1:1,000 and 1:10,000. The plates were incubated at 30° C. until growth occurred. When the samples kept at room temperature reached an acidity of 0.4 per cent and finally 0.5 or 0.6 per cent, they were again plated out as described above. This procedure in principle is similar to that of Ayers, Johnson and Mudge,<sup>15</sup> who feel that by plating out at various acidities one is more apt to get the characteristic lactic acid streptococci.

When the colonies had grown out, they were examined for the presence of lactic acid streptococci. These appeared as small, deep yellow or orange, smooth, raised and glistening colonies. A few were "fished off" and later con-

TABLE I  
MAJOR CULTURAL REACTIONS OF *S. fecalis* AS NOTED BY VARIOUS WORKERS

	VORPHOLOGY	HEMOLYSIS	HEAT RESISTANCE	BILE SOLUBILITY	ACTION ON MILK	INDOL	AESCULIN	GELATIN	GLUCOSE BROTH	GROWTH		SUCROSE	LACTOSE	INULIN	RAFFINOSE	MANNITOL	SALICIN	ARABUTIN	DEXTROSE	MALTULOSE	GLYCEROL
										10° C	45° C										
Callero <sup>7</sup> 1926	Pleomorphic cocci	-	+	-	Cong	-	-	-	-												
Went <sup>16</sup> 1926	Gm + oval diplo str	-	-	-	Acid no cong	-	+	after long time	-												
Gordon <sup>13</sup> 1922	-	-	-	-	-	-	-	-	-												
Grosmann <sup>11</sup> 1929	Oval or lancet	-	+	-	-	-	+	-	-												
Andrews and Horder <sup>15</sup> 1906	Short chained cocci	-	-	-	-	-	-	-	-												
Alsom <sup>17</sup> 1928	Oval pure, and short chains	-	60° C for 10 mins	-	-	-	-	-	-												
Meyer <sup>14</sup> 1928	Beta at times Gram <sup>+</sup>	-	+	-	-	-	+	-	-												
Sherrin and Stark <sup>21</sup> 1931	Gm + cocci oval	-	-	-	Red of lit mas before cong	-	-	-	-												
Bergey <sup>18</sup> 1940	Gm + cocci oval	-	-	-	Acid, cong	-	-	-	-												
Groves <sup>20</sup> 1924	Short chained cocci	-	+	-	-	-	-	-	-												

"Does not ferment various sugars"





firmed by Gram's stain, to be gram positive, diplococci, appearing in pairs and few very short chains. After several replatings the cultures were put in stock.

*Fecal Streptococci.* Samples of feces from both normal and sick human beings were obtained and a portion of the sample was emulsified in a tube of infusion broth and incubated at 30° C for a few hours. Bromthymol blue dextrose agar plates were streaked with some of this material and incubated at 30° C until growth appeared. The colonies of the fecal streptococci appear the same as the lactic acid streptococci on these plates.

Some of the material from the fecal specimens was placed in tubes of litmus milk and heated to 60° C for fifteen minutes. These tubes were then incubated at 30° C until acid production and coagulation occurred. From these tubes, bromthymol blue dextrose agar plates were streaked and incubated at 30° C until colonies of fecal streptococci appeared. This method is similar to those of Dible<sup>14</sup> and Ayers and Johnson.<sup>4</sup> A few of the cultures were obtained by streaking some of the fecal material directly on bromthymol blue dextrose agar plates and incubating them until colonies of fecal streptococci appeared. The cultures were replated several times to insure purity and then placed in stock.

#### EXPERIMENTAL

The purpose of this work was not so much to use new tests, as to utilize those recommended by the various workers mentioned in the beginning of this paper, to try to duplicate their results, and finally, to see what conclusions could be drawn from the assembled results.

Fifty cultures of the lactic acid and fifty of the fecal streptococci were selected for the experimental work. The following studies were made.

#### CULTURAL CHARACTERISTICS

It was impossible to differentiate between the two types of streptococci when grown in either infusion broth, 0.1 per cent dextrose infusion broth or on blood agar or bromthymol blue dextrose agar. Morphologically they were pleomorphic, varying in size and shape. The cocci were usually oval or lemon shaped, with an occasional round form. Generally there were no long chains produced, except by a few strains when grown in 0.1 per cent dextrose infusion broth.

Culturally, both types grew well on all four media mentioned above. On blood agar they varied in the type of hemolysis produced, the majority of the strains giving the Alpha type. All strains grew well on bromthymol blue dextrose agar, forming characteristic colonies.

Bromthymol blue dextrose agar was made according to the formula of Small and Kredler,<sup>22</sup> except that dextrose was used instead of lactose, and the pH was adjusted to 7.5. Dextrose seems to give a sharper colony and a deeper color. This medium is valuable in the primary isolation of members of either type of streptococci since they produce characteristic colonies. Other dextrose fermenters produce larger colonies and the coloration is not quite as deep yellow.

The cultures when grown in litmus milk, all produced acid. With the exception of six strains, they all reduced the litmus before coagulation. The

TABLE III\*

MORPHOLOGIC AND CULTURAL CHARACTERISTICS OF FECAL STREPTOCOCCI AND LACTIC ACID STREPTOCOCCI

NUMBER OF STRAINS	METHODS OF ISOLATION	MORPHOLOGY	HEMOLYSIS	GELATIN		HEAT RESISTANCE, 60° C. FOR 20 MINUTES	LITMUS MILK	REDUCTION OF AMMONIUM MOLYPDATE	REDUCTION OF JANUS GREEN	REDUCTION OF METHYLENE BLUE IN MILK	BILE SOLUBILITY	H <sub>2</sub> S PRODUCTION	ACID PRODUCTION AT 10° C.	ACID PRODUCTION AT 45° C.
				GROWTH 20° C.	LIQUEFACTION									
Fecal Str.	16	Plates streaked from broth emulsions	Oval cocci, pairs and short chains	Gamma (10) Alpha (6)	+	(2) + (14) -	(15) Typical (1) Atypical	(9) B (7) T	(2) ++ (7) + (7) -	(15) + (1) -	-	+	(4) 1.71 to 6.58 (12) -	4.18 to 4.28
	32	Heating and incubation in litmus milk	Oval cocci, no chains	Gamma (10) Alpha (22)	+	(4) + (28) -	(29) Typical (3) Atypical	(17) B (15) T	(3) ++ (14) + (15) -	(26) + (4) R (2) -	-	+	(4) 4.62 to 6.69 (28) -	4.15 to 4.40
	9	Direct plating	Oval cocci, no chains	Alpha	+	-	Typical	B	+	+	-	+	-	4.30 4.32
Lactic Acid Str.	35	1:100 and 1:10,000 dilution plated	Oval cocci, no chains	Alpha	+	(6) + (29) -	Typical	T	(31) ++ (4) +	(29) + (4) R (2) -	-	+	(31) 4.42 to 6.09 (4) -	4.10 to 4.66
	10	Plating direct on BTB agar	Oval cocci, pairs, few short chains	Alpha	+	(3) + (7) -	Typical	T	(6) ++ (4) +	(6) + (3) R (1) -	-	+	(4) 4.40 to 5.84 (6) -	4.25 to 5.64
Lactic Acid Str.	5	Streaking direct on BTB agar	Oval cocci, no chains	Alpha	+	(1) + (4) -	Typical (3) Atypical (2)	T	(3) ++ (2) +	(4) + (1) R	-	+	(6) 4.60 to 6.35	4.47 to 4.69

\*Reduction of Ammonium Molybdate.  
T, Blue color throughout the tube.  
B, Blue color only in butt of tube.  
R, Reduction of Janus Green.  
++, Red color.  
+, Purple color.  
-, Blue color.

Reduction of Methylene Blue in Milk  
+, Reduction of M. B. and coagulation.  
R, Reduction of M. B. only.  
-, M. B. not reduced.

typical reaction, acid, reduction of litmus but for a narrow pink band at the surface of the medium, and coagulation, was noted by Ayers and Johnson,<sup>4</sup> Sherman and Stark,<sup>21</sup> and others to be characteristic of either type of streptococci. Here it was noted that the lactic acid strains brought about this typical reaction much more rapidly than did the fecal strains.

TABLE IV  
SUGAR FERMENTATIONS

SUGAR	FECAL STREPTOCOCCI			LACTIC ACID STREPTOCOCCI		
	NO OF STRAINS PRODUCING ACID	pH RANGE OF ACID FORMERS	NO OF STRAINS PRODUCING NO ACID	NO OF STRAINS PRODUCING ACID	pH RANGE OF ACID FORMERS	NO OF STRAINS PRODUCING NO ACID
Arabinose	45	4.30-6.96	5	3	5.11-6.94	47
Dextrin	50	5.26-6.57	0	42	5.28-6.08	8
Dulcitol	0		50	27	6.69-6.86	25
Galactose	50	4.46-5.26	0	50	4.54-6.07	0
Glycerol	50	4.83-6.33	0	46	4.54-6.04	4
Inulin	37	6.53-6.96	13	28	6.33-6.96	22
Lactose	50	4.25-5.00	0	50	4.58-6.18	0
Levulose	50	4.08-5.07	0	50	4.30-5.12	0
Maltose	50	4.12-5.02	0	50	4.26-5.01	0
Mannitol	42	4.23-5.64	8	27	4.66-5.39	23
Raffinose	44	4.37-6.97	6	0	5.17-6.98	41
Salicin	50	4.13-5.18	0	34	4.35-5.00	16
Sorbitol	50	4.33-6.94	0	50	4.37-5.47	0
Sucrose	41	4.25-6.66	3	46	4.49-5.11	4

TABLE V  
FERMENTATION OF DEXTROSE

DROP IN pH NOTED EVERY OTHER DAY FOR A PERIOD OF THREE WEEKS

FECAL STREPTOCOCCI										LACTIC ACID STREPTOCOCCI									
3rd	5th	7th	9th	11th	13th	15th	17th	19th	21st	3rd	5th	7th	9th	11th	13th	15th	17th	19th	21st
4.24	4.00	4.00	4.00	4.00	3.96	3.96	4.00	4.03	4.07	3.98	4.15	4.15	4.10	4.07	4.17	4.17	4.20	4.12	4.26
5.26	5.03	5.03	5.03	5.03	5.03	5.03	5.07	5.07	5.12	6.18	6.18	6.18	6.15	6.10	6.10	6.10	6.05	6.05	6.01

The medium used for the fermentation studies was similar to that recommended by Ayers and Johnson.<sup>4</sup> The sugars were sterilized separately in 5 per cent aqueous solutions and then added to the tubes to make a final sugar concentration of 0.5 per cent. The cultures were incubated for seven days and the change in pH noted with the use of the quinhydrone potentiometer. Dextrose fermentations were tested every other day for a period of three weeks, to determine when the lowest pH was reached.

Ayers and Johnson<sup>4</sup> claim that *Streptococcus lactis* seems to reach a lower final pH than the fecal streptococci. It was found that the fecal strains produced a lower pH than the lactic acid groups and that they were more constant in their acid production, showing usually a smaller range in pH. A number of the lactic acid strains caused a rise in pH during their growth in some sugars, reaching in a few cases a pH of 6.02.

On the basis of group majority it was found that the fecal groups fermented arabinose, mannitol, raffinose and salicin. A very small majority of the lactic acid strains fermented mannitol and salicin and only a few strains fermented arabinose and raffinose. Dulcitol was not fermented by any of the fecal strains but by half (25) of the lactic acid strains. The remainder of the sugars as indicated in Table IV were fermented by nearly the same proportion of strains in either type.

In the fermentation of dextrose it was found that the lactic acid organisms generally reached a lower pH much sooner than the fecal strains. They also reverted back to alkaline sooner, and in three weeks showed a higher pH than the fecal strains.

Tests made to determine the amount of variation between parallel fermentations by the same strains, in regard to the drop in pH, showed that a difference of 0.03 to 0.09 of a pH unit could be expected. This difference was even greater in cases where the sugar was not readily fermented by the organism.

It is generally considered that *Streptococcus fecalis* will not grow at 10° C., although Ayers and Johnson<sup>4</sup> and Sherman and Stark<sup>21</sup> found that their strains did grow at that temperature. Here it is noted that when the strains were grown in dextrose infusion broth there were not very many which grew and produced acid at 10° C. This may have been due to two factors: namely, first, that the medium used was not a satisfactory one for the test; second, that under the conditions available it was impossible to maintain a constant temperature of 10° C., it usually varied from 6 to 8° C.

Sherman and Stark,<sup>21</sup> together with other workers, state that it is generally felt that *Streptococcus lactis* will not grow at 45° C., and that this test might be used as a means of differentiating the fecal and the lactic acid streptococci. It was noted here, however, that both types grew well and produced acid at 45° C., and further, that no distinction between them could be made on this basis.

In testing for the reducing ability of the strains, the three methods used by Ayers, Johnson and Mudge,<sup>19</sup> namely, reduction of Janus green, reduction of ammonium molybdate and the reduction of methylene blue in milk, were attempted.

Both types possess fairly strong reducing abilities, the majority of strains reducing the various dyes. In the reduction of Janus green the lactic acid strains seemed to have the greatest effect. However, in the reduction of ammonium molybdate, the condition was just the reverse, in that the fecal groups appeared to have the stronger reducing power. The reduction of methylene blue was fairly rapid in both types. The majority of the strains completely reduced the dye and coagulated the milk. A few strains of either type failed to reduce methylene blue or reduced it for a short period, after which the color came back throughout the tube.

In testing for heat resistance and bile solubility, the methods used by Alston<sup>17</sup> were followed. Bromthymol blue dextrose agar plates were used to grow the organisms after the exposure to 60° C. for the required time, since it was found that this medium served as a better guide to determining the numbers

surviving, than did blood agar plates. This was due to the fact that the medium turns yellow when acid is produced in large amounts.

The majority of strains were resistant to 60° C. for twenty minutes. However, in a few cases the organisms failed to withstand this temperature for a longer period than ten minutes. Ayers and Johnson<sup>4</sup> found that their strains withstood this temperature for thirty minutes.

All strains were insoluble in bile when tested in the three concentrations of 10 per cent sodium taurocholate, namely: 0.2 c.e., 0.1 c.e., and 0.05 c.e. to 1 c.e. of a twenty-four-hour broth culture. The pneumococcus controls were all soluble in these three amounts.

Tests for growth and liquefaction of gelatin showed that a few strains of either type were able to liquefy the gelatin. These strains were positive in three days and none of the other strains showed any liquefaction up to three weeks' incubation at 20° C. All strains grew well in this medium. The tubes were placed in the refrigerator before a final reading.

The production of hydrogen sulphide was noted by following the formula given by Ayers and Johnson.<sup>24</sup> All strains produced hydrogen sulphide when some sulphur compound was added to the basic medium. It was impossible to differentiate between the two types on this medium.

*Serologic Reactions.—Agglutinations:* Five strains of each type were selected for agglutination on the basis of their differences in the various tests as described above.

*Preparation of Monovalent Rabbit Serums:* An infusion broth was prepared containing 1 per cent peptone, to which was added 1.03 gm. of disodium acid phosphate and 0.12 gm. of potassium dihydrogen phosphate per liter. The reaction was adjusted to pH 7.6 and the medium dispensed in 40 c.e. amounts in small flasks, and sterilized at 15 pounds for fifteen minutes.

Forty-eight-hour cultures grown in this broth were centrifuged and resuspended in saline. This process was repeated several times. For use the cells from 40 c.e. of culture were suspended in about 25 to 30 c.e. of physiologic saline and killed by heating in a water-bath at 60° C. for one hour.

The rabbits were injected first subcutaneously on consecutive days, in 0.25, 0.5, and 1.00 c.e. amounts. A rest period followed, after which they received 0.25, 0.5, and 0.5 c.e. of each suspension intravenously. After a rest period of three or four days they received three more intravenous injections in just slightly larger amounts than previously used. This procedure was kept up for six weeks, each time injecting slightly larger amounts of the antigens, reaching a maximum of 2.0 c.e.

The serum of each rabbit was tested at intervals during the period of injections for agglutination titer. One week after the last series of injections the rabbits were bled from the heart, the serum collected and preserved in 0.3 per cent trikresol.

*Agglutination:* Tests were set up using homologous antigens, both with suspensions of live bacteria and those killed for the injections. It was found that the results were the same in either case; therefore, live suspensions were used throughout the remainder of the work.

The antisera were set up in dilutions ranging from 1:10 to 1:10,240, using 0.85 per cent NaCl for dilutions. To each tube, including a control, was added 0.5 c.c. of the homologous antigens. Controls of the various antigens and normal rabbit serums were set up along with the above tests. The tubes were placed in a water-bath at 52° C., read after three hours, and confirmed after eighteen hours in the water-bath. Cross-agglutination tests were set up to note whether there was any evidence of grouping. The titer of each serum, together with the results of the cross-agglutination tests, are shown in Table VI.

Both types are characterized by their ability to cross-agglutinate. This was noted by other workers, but especially by Hucker,<sup>26</sup> who found that the "serum of *Streptococcus faecium* (Orla-Jensen) agglutinated strains of *Streptococcus lactis*." It was noted that a division between these two types on the basis of agglutination tests would be difficult, hence agglutinin absorption tests were tried.

TABLE VI

RESULTS OF AGGLUTINATION AND CROSS-AGGLUTINATION TESTS WITH ANTISERUMS OF FIVE OF THE FECAL AND FIVE OF THE LACTIC ACID STREPTOCOCCI

	F 3	F 8	F 19	F 51	F 53	L 3	L 15	L 23	L 41	L 43	NORMAL RABBIT SERUM
F 3	5120	2560	2560	0	0	2560	160	0	1280	0	0
F 8	2560	5120	2560	0	0	0	0	0	1280	0	0
F 19	2560	2560	5120	0	0	0	0	0	1280	0	0
F 51	0	0	0	1280	0	0	0	0	0	0	0
F 53	0	0	0	0	2560	0	0	0	0	0	0
L 3	0	0	0	0	0	1280	160	0	0	0	0
L 15	0	0	0	0	0	0	2560	0	0	0	0
L 23	0	0	0	0	0	0	0	2560	0	0	0
L 41*	---	---	---	---	---	---	---	---	---	---	---
L 43	0	0	0	0	0	0	160	0	0	2560	0

\*L 41, one of the lactic acid strains auto-agglutinated continuously, hence no results could be obtained on its titer or on its cross-agglutinative ability.

*Agglutinin Absorption:* F 3, F 8, F 19, L 3, and L 15 serums were diluted 1:50 with a heavy suspension of each strain which showed cross-agglutination with them. These diluted serums were kept at 37° C. for four hours, with occasional shaking. They were then placed in the refrigerator overnight, after which they were centrifuged and the clear fluid used to set up agglutination tests with their homologous antigens. The change in titer was noted and recorded.

There were some evidences of absorption of agglutinins, although the test failed clearly to differentiate the types. F 3, F 8, and F 19 serums appeared to be the same in the cross-agglutination tests, but in the absorption reaction they show up as distinct variants with a common agglutinin. F 3, L 3, and L 15 possess a common agglutinin together with small amounts of specific agglutinins. L 15 seems to possess a predominating agglutinin which is also present in L 3 and L 43 in small amounts.

On the whole, the agglutination and the agglutinin absorption reactions were not of much value in attempting to show a clear distinction between the two types of streptococci. This is mainly due to the contradictory results obtained in cross-agglutination, and, perhaps, as stated by Hucker,<sup>26</sup> to a decided strain

specificity of some of the organisms. It is felt, however, that these strains are all serologically related, and that there exist several distinct variants.

Numerous attempts were made to secure an antigen of L41 which would not auto agglutinate, all of which were unsuccessful. The procedure recommended by Crowe,<sup>27</sup> was used in several attempts with some of the other strains which at times would auto agglutinate. This method, however, failed in the case of L14, which, if it showed no auto agglutination before the test was set up, always spontaneously agglutinated after incubation, as evidenced by the control tubes.

#### DISCUSSION

As noted in Tables I and II, there seem to exist some differences of opinion as to which reactions constitute the basic characteristics of these two types of streptococci. Variations in technique and tests used would tend to give some varied results. This perhaps accounts for the differences in opinion in regard to the relation of these two types of organisms. The types of media and the initial pH, it is felt, are two very important factors in attempting to correlate results when working with streptococci. A standardization of methods and technique would go far in eliminating many of the present discrepancies in respect to this field. Numerous workers have attempted this matter, and it remains now to select those which will bring out the important physiologic characteristics of the organisms.

These two types of organisms resemble each other very closely. There are slight differences but they may be attributed to the fact that the changes in environmental conditions would tend to bring about a change of some of the physiologic reactions, producing some of the variable characteristics which they possess. Their pleomorphic traits also suggest this.

Sherman and Albus, Jones and numerous other workers have shown that *Streptococcus lactis* is not an inhabitant of the udder and that it is not found in freshly drawn milk. It must enter the milk from some outside source, in very small numbers, for it cannot readily be isolated from milk less than thirty six to forty eight hours old. Since it is well adapted to a milk medium, it soon becomes the predominating organism and remains as such until the acidity becomes too great, when it again diminishes in numbers.

#### SUMMARY AND CONCLUSIONS

Fifty strains each of members of the fecal streptococci and the lactic acid streptococci were studied simultaneously for any morphologic, cultural, or serologic characteristics which would warrant their being classified as two distinct species.

It was the purpose of this study to assemble the most important methods and tests generally in use or those used particularly by various authors and note whether any differences in reactions occurred between these two types of streptococci. Variations in technique or new methods were employed only where it was impossible to carry out the original procedure.



Detailed studies were made on the growth and action in various media. Finally, immune serums were prepared by rabbit inoculation. Agglutination, cross-agglutination, and agglutinin absorption tests were set up.

It is felt that there exists no real difference between these two types of streptococci. Slight variations which occur are not of real importance since they may occur not only between the two types but also among the groups themselves. These slight variations perhaps are due to the fact that the organisms undergo extreme changes in environmental conditions.

Since the lactic acid streptococci appear to be able to withstand conditions as they occur in the animal body, it is reasonable to assume that they should be found in the intestinal tract. As indicated here and also by other workers, there seems to exist no real difference between the fecal streptococci and those normally found in milk. Therefore, it is felt that the *Streptococcus lactis* ingested with milk and some milk products is of the same type as that found in the feces. From here they may enter the soil where only the most vigorous can survive and in turn enter milk through sources of contamination.

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## THE QUININE TEST FOR HYPERTHYROIDISM\*

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WHILE the recognition of typical exophthalmic goiter or Graves' disease is devoid of difficulties, the many atypical cases in our midst require laboratory tests for confirmation of diagnosis. We are still puzzled by the large percentage of otherwise typical cases presenting no exophthalmos or no thyroid swelling. Indeed, in approximately 11 per cent of our series of cases both exophthalmos and goiter were absent at the time of the primary examination.

### TESTS FOR HYPERTHYROIDISM

Aside from the basal metabolism test, which, properly performed under ideal physical, mechanical, and psychological conditions, is still quite dependable in the majority of cases, there are many other tests that have been discussed in the literature from time to time. Among these are the adrenalin test, the Kottman test, the blood iodine and blood cholesterol tests, the erythrocyte sedimentation test, the velocity of the blood flow test, the impedance angle test, and the quinine tolerance test to which I called attention in 1920 and in later communications<sup>1 2 3</sup>. Many of these tests possess genuine clinical value. However, one need not go far afield to seek for reliable means of diagnosis of atypical exophthalmic goiter. In our experience the most dependable means are first, the experience and five senses of the doctor himself, second and third, the basal metabolic and the quinine tolerance tests.

*The Quinine Test*—Continuing our studies prior to 1920, we found in a series of over 4,000 cases of hyperthyroidism (with and without Graves' disease) that over 95 per cent tolerated from 30 to 90 gr of quinine sulphate daily for weeks without evidences of cinchonism. The relative immunity to cinchonism exhibited by these patients lent itself to the adoption of the quinine test for hyperthyroidism, the technique of which is as follows:

The patient is given a dozen capsules, each containing 10 gr (0.65 gm) of quinine hydrobromide (or the sulphate), with instructions to take a capsule 3 times a day. If after

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four days there are no evidences of cinchonism, the individual's reaction may be considered positive for hyperthyroidism. When 20 or 30 gr. (1.3 or 1.9 gm.) have been taken by persons in whom thyroid function is normal or deficient, symptoms of cinchonism develop. A sense of fullness in the head, impaired hearing with tinnitus, occasional dizziness and headache, and less frequently, gastric and bladder discomfort are experienced. If, however, the person is suffering with hyperthyroidism, the daily administration of 30 gr. of quinine may be continued with impunity for months, and often with distinct improvement in the clinical picture.

In our series 4.25 per cent were quinine negative. Forty-five presented varying degrees of quinine sensitivity. In the remainder the tolerance for quinine varied in dosage from 30 to 90 gr. a day for at least three weeks in each case.

Male patients predominated among those able to take the largest doses of quinine. Also, following the ingestion of the drug, there occurred in at least 60 per cent of this series such evidences of general improvement as increased weight, lessened excitability and tremor, improved sleep, and a reduction in the heart and basal metabolic rate. The advantages of quinine in treatment were outlined in another contribution.<sup>4</sup>

The merits of the quinine test are its simplicity and relative dependability. The results are not influenced by emotionalism nor are such factors as transient nervousness and errors in technic involved. Moreover, the test requires no "basal conditions" for its use, the patient requiring no preliminary period of rest or starvation.

The quinine test does not discriminate between toxic adenoma and exophthalmic goiter. This differentiation requires not laboratory but clinical deductions. In a general way, however, we feel that the average patient with exophthalmic goiter is somewhat more tolerant to quinine than is the average sufferer from toxic adenoma.

TABLE I

TOLERANCE FOR QUININE IN 4,000 CASES OF HYPERTHYROIDISM WITH AND WITHOUT GRAVES' DISEASE

Quinine Positive:		
Patients tolerating 30 gr. daily	1,334 or	33.4 per cent
Patients tolerating 40 gr. daily	836 or	20.9 per cent
Patients tolerating 60 gr. daily	725 or	18.1 per cent
Patients tolerating 80 gr. daily	527 or	13.2 per cent
Patients tolerating 90 gr. daily	408 or	10.2 per cent
Quinine Negative:		
Patients sensitive to quinine	45 or	1.1 per cent
Patients tolerating 20 to 30 gr. daily	125 or	3.1 per cent
Total	4,000 or	100.0 per cent

#### QUININE NEGATIVE CASES

It appears that in at least 95 per cent the element of hyperthyroidism must be present in order that the quinine test be positive and a relative immunity to cinchonism be expected. In other words, patients able to tolerate 30 or more grains of quinine hydrobromide or quinine sulphate a day for a few days or longer present the most important evidence of hyperthyroidism, namely, a

*heightened basal metabolism rate* This was emphasized when it was observed that, barring the occasional instance of quinine sensitivity in this series, most of the patients with mild evidence of emetionism (i.e., slight fullness in the head and tinnitus) were sufferers from the uncommon type of Graves' disease with a normal basal metabolism rate. Of late years clinicians who see many cases of Graves' disease realize that a heightened metabolic rate is occasionally conspicuous by its absence in an otherwise typical syndrome. Despite heart hurry, trembling, loss in weight, exophthalmos, emotionalism, and nervousness, in some instances of active Graves' disease the element of hyperthyroidism per se may not be demonstrable. In our experience approximately 15 per cent of subjects of Graves' disease present no rise in metabolism and are likely to be quinine negative. Among these are included cases of thyroid quiescence due to remission of the syndrome following thyroidectomy, roentgen ray treatment, iodine administration, or as a result of spontaneous involution. While exophthalmos, heart excitability, tremor and fatigability may still prevail to an extent, the thyroid gland is not in a state of hyperactivity.

#### QUININE TOLERANCE BY THYROID NORMAL PERSONS

The tolerance to quinine by sufferers from malaria and from such febrile conditions as pneumonia, acute tonsillar diseases, and other infectious processes must not be construed as a state of natural immunity from the effects of the drug, but as a temporarily altered condition of the bodily reactions, to return to normal on the recovery of the patient. These instances of tolerance are easily determined and need not detract from the reliability of the quinine test in hyperthyroidism. In quinine negative persons it is found that after taking 2 or 3 capsules of the quinine salt each containing 10 gr. at approximately five hour intervals, the typical head and ear symptoms of emetionism develop, indicating intolerance to this dosage. In order to ascertain the amount of quinine tolerated by normal controls we succeeded in enlisting the services of eight apparently normal adults of average size and weight, varying in age between twenty and forty one years. Four were men and four were women. The period of observation was two weeks in each subject. Head and ear symptoms were indices of intolerance, and quinine sulphate was given in evenly divided doses five or six hours apart, t.i.d. Two subjects were able to tolerate 12 gr. daily, three could take but 9 gr. a day, two could not exceed 6 gr., and one developed emetionism when the dosage exceeded 3 gr. daily. According to this group the average normal person can tolerate 8.25 gr. of quinine sulphate a day for two weeks without experiencing symptoms of emetionism.

#### VARIATIONS IN QUININE TOLERANCE IN THE SAME PATIENT

In the average case of hyperthyroidism quinine tolerance appears to vary directly with the severity of the symptoms. The degree of tolerance runs fairly parallel with the heightening of the basal metabolism rate. Early or mild cases usually tolerate 30 gr. or more of quinine hydrobromide or sulphate daily in the presence of a basal metabolism rate varying from plus 30 to plus 40 per cent.

In the average sufferer with a metabolic rate of plus 50 or over, and during crisis, quinine tolerance may be increased to even 90 gr. daily and, in a few instances in our series, even higher.

When, while under treatment, a patient formerly tolerant to large doses of quinine now begins to complain of head and ear symptoms, it is invariably found that the basal metabolism rate, too, is approaching or has become normal. Formerly capable of tolerating large doses of quinine daily, such a patient now gradually becomes quinine-negative. The degree of quinine tolerance manifested by the individual is a fairly satisfactory index of the degree of thyroid hyperfunction. While precise figures have not yet been worked out, further investigation may determine the quinine tolerance of a given subject of hyperthyroidism to be quite as reliable an index of the presence or severity of the disease and of the progress toward recovery as is the basal metabolic rate.

Relative immunity to cinchonism obtains only during the time the individual is suffering from the disease, the amount of quinine satisfactorily tolerated by the patient running fairly parallel with the height of the basal metabolic rate. When, having been capable of taking 30 or more grains of quinine hydrobromide or sulphate with impunity for weeks or months, the patient complains of buzzing in the ears and roaring in the head, it will be found that the heart and metabolic rates and sense of well-being have become quite normal. At this point those clinicians employing the drug may continue giving quinine in doses tolerated without cinchonism, which in the average individual may vary from 3 to 10 gr. a day. This dosage is suggested for two or three months, when it may be discontinued.

#### SENSITIVITY TO QUININE

Sensitivity to cinchonism and its derivatives may occur where least expected, so that when administering quinine either as a test or as a therapeutic measure, the medical attendant should see the patient daily for the first few days. In our series there were patients in whom intolerance was associated with more or less troublesome symptoms, particularly referable to the skin.

Cases of sensitivity to quinine can be apprehended with the Boerner test prior to quinine ingestion. A positive Boerner's reaction probably means that the patient will have discomfort from the administration of the drug; a negative reaction, on the other hand, does not preclude the occurrence of urticaria. Patients presenting an idiosyncrasy to quinine may be found tolerant to quinidine. Boerner, himself sensitive to quinine, describes a positive test as follows:<sup>5</sup>

The method of conducting the test is similar to the cutaneous tuberculin test (von Pirquet) with quinine sulphate as antigen. The forearm is cleansed with alcohol and dried. Two abrasions are made with a needle about 3 inches apart. The scarifications are just deep enough to remove the superficial layers of the skin. To one of these abrasions, powdered quinine sulphate is applied. The other is left untouched to show the amount of irritation produced by the traumatism. In about five minutes the abrasion to which the quinine has been applied begins to itch slightly. This is followed by slight edema along both sides of the pin mark which in ten minutes becomes very pronounced. At the end of fifteen minutes the reaction is at its height, and at this time is surrounded by a zone of erythema presenting a mottled appearance and extending over an area about 1 inch in diameter. Instead of powdered quinine sulphate, a solution of quinine bisulphate in 1:10 dilution with normal salt solution may be used.

## CONCLUSIONS

1 From our observations on a series of over 4,000 cases it appears that the quinine test for thyrotoxicemia is a dependable guide in diagnosis, the frequency of error not exceeding 5 per cent. As with basal metabolic studies, the test does not discriminate between toxic adenoma (true hyperthyroidism) and exophthalmic goiter (Graves' disease).

2 The tolerance for quinine by subjects of hyperthyroidism appears to vary in direct proportion with the height of the basal metabolism rate and is fairly parallel with it, serving as a guide in progress under treatment. Depending upon the severity of active hyperthyroidism, patients are capable of taking 30 or more grains of quinine sulphate or hydrobromide daily for weeks without evidence of cinchonism.

3 In the occasional instance of a quinine negative subject who was an otherwise typical case of exophthalmic goiter, it was discovered that we were dealing with an uncommon case of Graves' syndrome, apparently without the element of hyperthyroidism. In these patients, despite nervousness, sweating, wasting, exophthalmos, heart hurry, trembling, etc., the basal metabolism rate remained within normal limits.

4 It appears that the quinine test is as dependable as the basal metabolism rate and as accurate a guide in treatment, it has the advantage of requiring no costly apparatus in its performance nor does it require "basal conditions" of starvation and rest.

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# CONGENITAL THINNING OF THE WALL OF THE RIGHT ANTERIOR AORTIC SINUS OF VALSALVA\*

ANTERIOR INTERVENTRICULAR SEPTAL DEFECT (PROBABLY BULBAR SEPTAL),  
SLIGHT DEXTROPOSITION OF THE AORTA AND BACTERIAL ENDOCARDITIS

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A CASE of congenital thinning of the wall of the right anterior aortic sinus of Valsalva, anterior interventricular septal defect and dextroposition of the aorta, terminating with bacterial endocarditis, is the subject of this report. A close correlation was established between the embryologic development of the heart and the morphology of the defects on the one hand and between the autopsy findings and the clinical manifestations elicited by ordinary methods on the other. It is of additional interest and significance that the right orifice of the interventricular septal defect occupied a position behind the septal cusp of the tricuspid valve. This location would ordinarily have invalidated the classification of this defect as being bulboseptal in type had it not been for the dextroposition of the aorta, the typical situation of the left ventricular ostium of the defect and the associated congenital thinning of the wall of the right aortic sinus of Valsalva.

## CASE REPORT

S. M., white male, aged sixteen years, complaining of headache, weakness, hematuria, and soreness of the left arm and leg of a week's duration, died the day following admission to the hospital (service of Dr. Thomas McCrae). There had been no history of sore throat, growing pains, chorea, or of acute rheumatic fever, and there had been no cyanosis, dyspnea, or edema. A chance physical examination at the age of two years revealed that the heart was not normal and the parents of the child were advised to restrict his exercise. Following admission, the third left toe became painful, red and discolored, a right facial palsy and flaccid paralysis of the left arm and right side of the tongue developed and petechiae appeared in the conjunctivae, and on the tip of the right index finger. On physical examination there was limited expansion of the left upper thorax and the left diaphragm did not appear to move as freely as the right. The cardiac impulse was widespread, wavy, diffuse, and rapid with the apex beat in the sixth left interspace, 13.5 cm. from the midsternal line. The right border of cardiac dullness extended 4 cm. from the midsternal line and a systolic thrill was palpable over the lower part of the thorax. On auscultation a loud systolic murmur was heard over the entire precordium transmitted into the neck and left axilla with its maximum in intensity at the third interspace, to the left of the sternum. The temperature was 100° F, the pulse rate 110 per minute, the respirations 28 per minute, and the blood pressure 130 mm. mercury systolic and 90 mm diastolic. The blood contained 80 per cent Hg, 4,000,000 erythrocytes, and 16,700 leucocytes; *Staphylococcus aureus* grew in blood culture. The urine contained blood and albumin.

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## POSTMORTEM EXAMINATION (2 HOURS AFTER DEATH)

The combined gross and microscopic diagnoses were (1) Congenital heart disease, anterior interventricular septal (probably bulbar septal), slight devtoposition of the aorta and bacterial endocarditis and congenital thinning of the wall of the right anterior aortic sinus of Valsalva, (2) subaortic stenosis, (3) endocardial systolic pocket of the left ventricle, (4) fenestration of posterior aortic cusp (5) marked endocardial sclerosis, especially of the aortic cusps, (6) acute bacterial endocarditis of the aortic cusps and a portion of the endocardium of the right ventricle opposite the orifice of the interventricular septal defect, (7) septic infarction of the brain kidneys, spleen, lungs, and third toe of the left foot, (8) widely distributed cutaneous, mucosal, and serosal petechial hemorrhages



Fig 1—Heart showing interior of left ventricle mitral leaflets aorta and interventricular septal defect (probe) The left aortic cusp is shaped like a cup lies above the anterior leaflet of the mitral valve and presents a darkened area of thrombus formation The posterior aortic cusp is markedly thickened and fenestrated in its left portion The interventricular septal defect lies below the commissure of the right and posterior aortic cusps A systolic endocardial pocket is situated upon the muscular prominence of the interventricular septum

Postmortem bacteriologic studies *Staphylococcus aureus* was recovered in pure culture from blood and from the substance of a splenic infarct

## DESCRIPTION OF HEART

The heart was large, showing hypertrophy of the walls of both ventricles, and sclerosis of the endocardium of the left auricle, mitral leaflets, aortic cusps, and subaortic area (Fig 1) The superior portion of the interventricular muscular septum was unusually well developed and caused a slight degree of subaortic stenosis The thick gray, opaque endocardium of this region formed a systolic endocardial pocket on the most prominent portion of this muscular ridge, the opening of the pocket being directed downward toward the apex of the ventricle The aorta was rotated slightly backward and to the right and was continuous with the somewhat contracted chamber of the left ventricle which was traversed near its apex by a fibrous band resembling a chorda tendineae The aortic cusps were thickened, nodular, adherent to each other and irregular in size and shape with inverted margins and



broad retracted stubby commissures. The left aortic cusp was situated upon the anterior leaflet of the mitral valve and presented a euplike appearance with a wide base and narrow constricted orifice; there was a small oval, roughened plaque of fresh thrombotic material on the ventricular aspect of this cusp. On the lower part of the commissure of the right and posterior aortic cusps the endocardium was covered by a small amount of thrombotic material. Directly below this area and behind the superior ledge of the subaortic muscular shelf was an oval, funnel-shaped interventricular septal defect. This was lined with thickened endocardium and bordered anteriorly and inferiorly by muscular tissue while posteriorly it approached to within 1 cm. of the pars membranacea.

In the dilated right ventricle (Fig. 2) the septal defect presented a larger vertical, slitlike orifice beneath the septal insertion of the anterior tricuspid ledge and above and

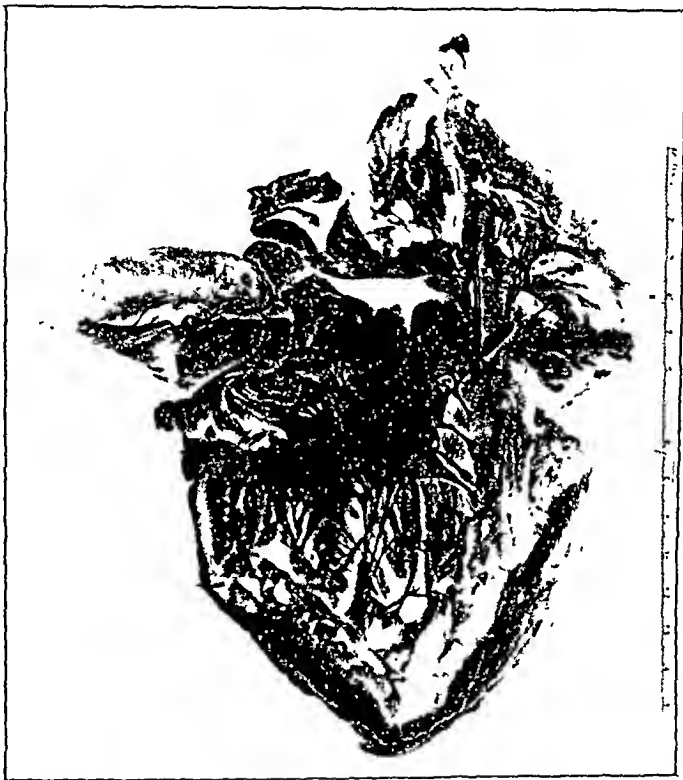


Fig. 2.—Heart showing interior of right auricle and ventricle. The orifice of the interventricular septal defect opens behind the septal cusp of the tricuspid valve (probe). An area of mural endocarditis can be seen on the anterior tricuspid ledge directly above the probe.

posterior to the extremely small papillary muscle of Lancisi. A globular thrombus with a fan-shaped base radiated outward from the right orifice of the defect along the endocardium of the posterior midportion of the anterior tricuspid ledge. Above and anterior to the septal defect was a thin, diamond-shaped, membranous septum which separated the pulmonary conus from the aorta. The two superior margins of this septum were formed by the converging borders of the right and left pulmonic cusps while the inferior margins were bordered by two muscular bands of the conus. These latter extended downward to the papillary muscle of Lancisi and to within 0.6 cm. of the anterior margin of the interventricular septal defect, where they met to form the inferior angle of this area of congenital thinning. The aortic surface of this septum lay opposite the right anterior sinus of Valsalva.

The descending branch of the left coronary artery was narrowed at about the junction of the upper third and lower two-thirds by an inflammatory lesion lying deep in the epicardial

fat, the caliber of its lumen was further diminished at this point by a small amount of thrombotic material. Distal to this lesion the epicardium was dotted over a fan shaped area with small petechial hemorrhages. The right coronary orifice was situated at the extreme right superior portion of the sinus of Valsalva, well above and to the right of the area of congenital thinning.

**Weights and Measurements** The heart weighed 500 gm and measured 15 by 10 by 7 cm.

The thickness of its walls was as follows: left ventricle, 3 cm, right ventricle, 0.8 cm, each auricle, 0.2 cm. The circumference of the valve orifices were aortic, 6.0 cm, pulmonary, 5.5 cm, mitral, 9.0 cm, and tricuspid 10.5 cm. The depth of the ventricles measured, from the base of the semilunar cusps to the apex of the chamber, left 8.5 cm and right 10.0 cm. The left ventricular orifice of the interventricular septal defect was situated 1.5 cm behind the edge of the subaortic muscular shelf and had a diameter of 0.4 cm. The right ventricular orifice was 0.5 cm in height while the defect itself was about 1.0 cm in length. The diamond shaped area of congenital thinning in the conus measured 2 by 1.5 cm.

### MICROSCOPIC DESCRIPTION

**Wall of the Right Ventricle Opposite Interventricular Septal Defect**—Sections from this area disclosed numerous small excrescences on the endocardial surface, composed chiefly of masses of conglomerated platelets and large numbers of cocci occurring singly and in small clumps. Underneath these was a wide layer composed of erythrocytes, fibrin, polymorphonuclear leucocytes and mononuclear phagocytes, many of which were necrotic. The fibrin at the base of this zone filled the interstices between the thick collagenous fibers of the endocardium, which beyond the lateral limits of the thrombus consisted of a wide zone of compact relatively vascular scar tissue. Immediately underneath the area of thrombus formation the myocardium was replaced by young granulation tissue.

**Area of Thrombus Formation in Descending Branch of Left Coronary Artery**—The deeper half of the vessel wall, lying in epicardial fat was surrounded and its adventitia and outer portion of its media destroyed by a large abscess which showed two or three discrete areas of necrosis near the center and an area of granulation tissue about the periphery. The intima of the coronary artery in this situation was covered with thrombotic material. Several small localized inflammatory lesions and hemorrhages were noted in the neighborhood of the large abscess especially on the surface of the pericardium.

**Left Aortic Cusp**—This was markedly thickened by old hyalinized connective tissue. About the midportion of the ventricular aspect of the cusp there was a large area of ulceration which extended through approximately three quarters of the thickness of the cusp. The margins were undermined and the floor of the ulcer was covered by thrombotic material. The surface of the lesion contained dense colonies of cocci. The surrounding area was densely infiltrated with inflammatory cells, chiefly polymorphonuclear leucocytes which had coalesced in several places to form small abscesses, causing additional destruction of the unperforated portion of the cusp.

**Endocardial Pocket**—This consisted of an endothelial lined, cusplike, hyaline structure in which a few shrunken fibroblasts remained. Where it arose from the heart wall, the fibers composing the cusp ran parallel with the endocardial surface. In the valvular portion proper, however, the axis of these fibers changed abruptly so that they ran in a direction perpendicular to the endocardial surface except at the extreme tip where they terminated in a whorl formation.

**Myocardium**—There were innumerable, scattered, small, focal inflammatory lesions composed of necrotic muscle fibers, mononuclear phagocytes, polymorphonuclear leucocytes, lymphocytes and plasma cells in about the proportion named. These lesions were distributed in relation to vascular structures. The small arteries were generally thickened and embedded in perivascular fibrous tissue, their walls were edematous and their lumens narrowed and some times obliterated. Several areas in the myocardium were replaced by dense scar tissue in which a few atrophic muscle fibers were present.

**Aorta**—The arterial branches of the vasa vasora of the adventitia were frequently thickened. There were several small necrotic and granulating foci in the media and adventitia.

## DISCUSSION

The anatomical basis for the defects present in this case appears to consist of an imperfect resolution of the bulbus cordis manifested by an incomplete and defective formation of the aortic septum associated with partial failure of spiral rotation. The bulbus cordis of the fetal heart is a muscular structure lined by spirally arranged endocardial swellings, the proximal and distal pairs of which are early recognizable. The situation of the former marks the division of the pulmonary conus from the right ventricle, while the latter corresponds to the site of the future semilunar cusps. Both unite with the descending septum aortiepulmonale to form the aortic septum which, when completed, divides the pulmonary artery from the aorta and also completes the division of the primitive heart by forming the upper and anterior interventricular septum (bulbar septum) in the region of the pulmonary conus. At one phase of the development the septum aortiepulmonale has descended from above while the distal and proximal bulbar swellings have fused in the midportion and below, thus temporarily permitting two openings to connect the two arterial trunks. The proximal opening lies below the semilunar valves and leads from the aortic into the pulmonary conus; its persistence in this case resulted in the interventricular septal defect. The distal opening in the aortic septum lies behind an aortic sinus (most frequently the right) and leads into the conus of the right ventricle between or beneath the pulmonary cusps. The homologue of this latter opening is normally found as the foramen Panizzae in the crocodile (Abbott<sup>1</sup>). In human beings, malformations in this region provide the embryologic basis for congenital lesions of the right aortic sinus of Valsalva. In our case the thinning of the bulbar aortic septum probably represented a defective formation of connective tissue obliterating the opening. Aneurysms with or without rupture (Abbott<sup>2</sup>) and a failure of the opening to become obliterated (Rickards<sup>3</sup>, and Charteris<sup>4</sup>) are related lesions with an identical embryologic basis.

The dextroposition of the aorta can be accounted for on the basis that the aortic septum failed to undergo a complete spiral rotation. This dextroposition was of additional interest and significance in that it was responsible for two other features. The first of these concerns the right orifice of the bulboseptal defect which opened behind the septal cusp of the tricuspid valve. After examining the specimen Abbott<sup>5</sup> stated that were it not for the typical situation of the left ventricular ostium and for the associated congenital thinning of the wall of the right aortic sinus, this unusual situation of the right orifice would invalidate the classification of the defect as being bulboseptal. The second result of the dextroposition was the formation of a subaortic stenosis by a prominent muscular shoulder derived from the interventricular septum directly below the aortic orifice. That a stenosis existed in this region is further indicated by the presence of an endocardial pocket upon the subaortic muscular prominence. Although the congenital origin of these pockets has been suggested by Sotti,<sup>6</sup> the consensus of opinion (Kaewel,<sup>7</sup> and Ziegler<sup>8</sup>) is that they are acquired lesions due to mechanical or inflammatory factors. Depending upon the direction of their opening these struc-

tures have been designated by Kiasso<sup>9</sup> as diastolic and, Saphir<sup>10</sup> especially, has emphasized their importance as indicating morphologic evidence of functional conditions within the heart

#### CLINICAL PATHOLOGIC CORRELATION AND SUMMARY

The clinical events which overhang a patient with congenital defects of this type are bacterial endocarditis and aneurysmal dilatation with rupture of the aortic sinus. The lesions of bacterial endocarditis tend to occur about the opening of the defect and secondarily upon the walls of the right ventricle where the repeated impaction of the shunted blood results in a localized area of lessened resistance. The aortic valve in this case was the seat of an old sclerosing lesion upon which an acute bacterial endocarditis was superimposed. The infected blood from the left ventricle was shunted through the bulboseptal defect into the chamber of the right ventricle where its impaction upon the outer wall resulted in extensive mural endocarditis, the infected thrombi in the ventricular chambers provided the source of embolic abscesses which were found in the organs of both the lesser and greater circulations. The only evident source of infection for the bacterial endocarditis was a slight nasal sinusitis which we regard as of doubtful importance. The hematuria was due to multiple infarcts of the kidneys while the headache and paralysis resulted from similar disturbances with abscess formation and hemorrhage in the right cerebral hemisphere with suppurative involvement of the meninges. The clinical evidence of areas of infarction involving the extremities provided the diagnostic clue to the acute thrombotic lesions within the chambers of the heart and embolic occlusion of arterial branches in the brain and kidneys. The presence of significant physical signs, the systolic thrill and harsh murmur heard best at the third left inner space and unassociated with cyanosis led to the clinical diagnosis of interventricular septal defect. The indurated inflamed area about the left coronary artery coupled with the presence of thrombotic material in the lumen of the vessel was regarded at the autopsy table as probably indicating embolism of that vessel. A study of the microscopic sections from this area, however, made it apparent that an abscess in the epicardial fat was the primary lesion, the thrombosis of the vessel being a secondary feature.

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## STUDIES ON ANTHRAX\*

### CLINICAL REPORT OF TEN HUMAN CASES

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THE cases of anthrax to be reported in this paper occurred in a Delaware County mill which handles goat hair imported from the Orient (China and India), for the manufacture of inner-lining. This mill has been in operation for over twenty years, and not until March, 1933, has there ever been reported a case of anthrax. Since then, four cases occurred among workers attached to the carding room, where the compressed bales of hair are taken apart and the hair is dusted out and carded. One case occurred in a woman who worked in the weaving room, handling hair bobbins. She was married to a man who had had anthrax and had recovered from it several months before. Two of the patients were children who lived in the mill-town. Their houses were located directly behind the carding room. The boys had never been within the mill's buildings; however, they played ball near the ground where waste hair from the carding machines was burned. Their mothers worked in the weaving department of the mill. The eighth case occurred in a Philadelphia plant which obtained its hair bobbins from the local mill by truck.

All the cases were studied in the Chester Hospital, and the clinical diagnosis of anthrax was confirmed in each case by smear and culture.

It is obvious that in spite of the present regulations governing the importation of hair, some infected material got to this mill. An attempt was made to determine the source of infection, and various samples of hair, taken at random, were studied bacteriologically by Dr. George Sickel of this city. Anthrax bacilli were recovered from one sample, on culture and guinea pig inoculation. Several months later the tests were repeated. Samples of dust and waste hair gotten from carding machines A and B, and a mixture of the same material obtained from the dust box, were examined bacteriologically.

On culture, a prolific overgrowth of contaminating organisms made the search for anthrax bacilli impossible. The samples were each placed in a glass funnel lined with gauze, and 100 c.c. of neutral plain bouillon were

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poured over it. The material filtered through was collected and injected into a series of guinea pigs. Only the animals injected with the dust box sample filtrate died of anthrax (Table I).

ANIMALS GUINEA PIGS	DOSE SUBCUT	DATE	RESULTS
1	$\frac{1}{2}$ cc	4/6/34	Dead 4/ 9/34 Positive anthrax
2	$\frac{1}{2}$ cc	4/6/34	Dead 4/10/34 Positive anthrax
3	$\frac{1}{2}$ cc	4/6/34	Dead 4/10/34 Positive anthrax
4	1 cc	4/6/34	Dead 4/ 8/34 Positive anthrax
5	1 cc	4/6/34	Dead 4/ 8/34 Positive anthrax
6	1 cc	4/6/34	Dead 4/ 8/34 Positive anthrax

Heart blood cultures of each guinea pig made on nutrient agar slants and on tubes of nutrient bouillon incubated twenty-four hours at 37.5° C. showed anthrax growth. Smears made from the cultures and examined microscopically revealed anthrax organisms.

The growth on agar slant was emulsified with 5 cc of normal saline solution. One cubic centimeter of this emulsion was then diluted with 40 cc of normal salt solution and 0.5 cc of this solution was injected into each of two rabbits (Table II).

RABBITS	DOSE SUBCUT	DATE	RESULTS
1	0.5 cc	4/13/34	Dead 4/15/34 Positive anthrax
2	0.5 cc	4/13/34	Dead 4/16/34 Positive anthrax

Heart blood cultures and smears were made from each rabbit. They revealed anthrax organisms.

As it is seen from these results the anthrax organism present in this mill and which caused the cases of anthrax to be reported in this paper was a very virulent strain.

We were also interested in determining whether the water before and after use in the various buildings became contaminated, since it drained into a nearby stream. Both on culture and animal inoculation (six guinea pigs were each injected subcutaneously with 5 cc of the water to be studied) the various water samples proved negative for anthrax. The tests were repeated twice, two weeks apart.

#### REPORT OF CASES

CASE 1.—A B. P. white male eighteen years of age, was seen on March 25, 1933, when he stated that two days before he had scratched his face while at work in the carding room. A typical lesion, the size of a coat button, was found on the right upper neck. The indurated area of painless edema was over three inches in diameter. The lymph nodes were not palpable. On admission to the Chester Hospital, the temperature was 101° and pulse 90.

Following the local administration of serum given according to the technique of Regan,<sup>1</sup> there was marked spread of the swelling. On March 26 the face and neck were markedly edematous. At 1.00 p.m. a blood culture was taken and proved to be sterile. The edema spread to the chest, and by the following morning, it extended down to the costal margin. The skin showed a bluish mottling. The spleen was not palpable. The patient complained of tension and pressure within the swelling. On the twenty-seventh the face was distorted. The

lesion consisted of a black ulcer half a centimeter in diameter with a surrounding vesicular ring, and a zone of redness. The temperature dropped to normal, while the pulse rate was very rapid.

Dr. Lucchesi of the Philadelphia Municipal Hospital, who was called into consultation, advised the administration of a large dose of serum (300 c.c.). The same night the patient became restless and delirious. The skin of the neck split along the lines of cleavage. The temperature rose to 102.6°. The patient died on March 28, 1933, at 4:00 A.M., on the fifth day of the disease.

TABLE I

## CASE 1

DATE	TIME	SERUM	ROUTE	REACTION	REMARKS
3/25/33	3:00 P.M.	85 c.c.	Intraven.	T. 103°	Swelling spread involving neck and jaw. W.B.C., 4,400; Polys, 62 per cent
		15 c.c.	Locally	P. 114	
	10:00 P.M.	50 c.c.	Intraven.	None	Further spread of edema
		15 c.c.	Locally		
3/26/33	9:00 A.M.	30 c.c.	Intraven.	T. 100°, P. 100	1:00 P.M. Blood culture sterile
	2:00 P.M.	50 c.c.	Intraven.	T. 100°, P. 100	
	11:00 P.M.	50 c.c.	Intraven.	T. 103°, P. 140	
3/27/33	10:00 A.M.	50 c.c.	Intraven.	T. 103°, P. 140	Neck, face, chest markedly swollen Patient toxic, pulse rapid, delirious
	8:00 P.M.	300 c.c.	Intraven.	T. 103°, P. 140	

An autopsy was performed by Dr. George Sickel six hours after death. The positive findings were as follows: Small black eschar was found on the right side of the neck just below the mandible. Extending from it over the lower face, neck, and chest to the costal margin, the subcutaneous tissues were the seat of a very marked and peculiar gelatinous edema. The chest wall was 6 to 7 cm. thick, and its cut surface resembled that of gelatin. A serous fluid ran out from the dependent portion of the incision. It was not hemorrhagic and did not contain gas. The cut vessels did not bleed, and in the subcutaneous tissue, they appeared as orange yellow projections as if they were thrombosed. The lungs did not show any pathologic change, except for an old healed primary tubercle. The heart was normal except for a few petechial spots on the anterior surface of the ventricles near the atrio-ventricular groove. The abdomen showed normal relations. The liver only showed loss of normal mottling. The spleen was but slightly enlarged and fairly firm. Its cut surface showed very prominent malpighian bodies. It did not resemble the soft splenic tumor of acute infection. The stomach showed a large ragged hole in the posterior wall at the cardiac end and just below the esophageal opening. There were no inflammatory reactions around this opening and surrounding peritoneum (probably due to postmortem digestion). The lymph glands were not enlarged. Blood obtained from the heart as well as smears from the spleen showed on culture anthrax bacilli. This organism was injected into a guinea pig by the subcutaneous abdominal route, with the production of a similar gelatinous swelling and death within forty-eight hours. Smears from the guinea pig's spleen showed anthrax bacilli.

Microscopic study showed the following:

*Human Sections.*—The heart showed cloudy swelling and edema; some capillaries were fairly full but not distended. The lung showed marked swelling of the interalveolar septa, caused largely by greatly distended capillaries plus mononuclear cell infiltration. The alveoli were distended, but showed no exudate. In some areas they contained a few red blood cells. The spleen did not show the engorgement seen in other acute infections. The malpighian bodies were enlarged and appeared to show an excessive number of large mononuclear cells. The pulp was fairly well filled with red blood cells and lymphocytes. The liver showed marked changes ranging from cloudy swelling to complete necrosis. The latter was widely and irregularly distributed, involving large areas. The capillaries were here and there, well filled with red cells, but in most areas the spaces between liver cords were filled with granular debris. In the kidneys, the glomerular tufts were well distended and in some areas the

intracapsular spaces contained a granular material. The tubular epithelium showed extensive degeneration and desquamation, with congestion of the intertubular capillaries. The subcutaneous tissue from the chest wall, at the level of the diaphragm, showed a very intense edema and cellular infiltration, mostly of polymorphonuclear cells, with a fair number of lymphocytes.



Fig 1—Section of guinea pig heart. High dry lens. Approximately 440 diameters magnification. Note great many clumps of anthrax bacilli in the blood clot attached to the endocardium. Many organisms are seen within and outside of blood vessels of the myocardium (Lillie's stain).

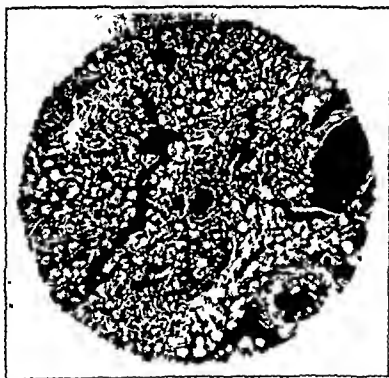


Fig 2—Section of subcutaneous tissue of chest wall of human case. High dry lens. Approximately 440 diameters magnification. Anthrax bacilli are shown in great abundance in tissue spaces (Lillie's stain).

*Guinea Pig Sections*—All the organs showed the same type of reaction as described for the human sections, except that it was much more intense. The capillaries were congested throughout, being filled with red cells and anthrax bacilli, but they did not give one the impression of being blocked. Actually, the smaller arteries appeared more occluded than the



capillaries. While the guinea pig sections stained by hematoxylin-eosin showed anthrax bacilli, these are much better shown by Lillie's modification of Gram's stain.<sup>7</sup> Great masses of bacilli were seen in the heart's blood, lung, spleen, etc. (Fig. 1). In the human sections careful scrutiny detected anthrax bacilli in the subcutaneous tissue of the chest and the spleen. An occasional organism was found in the renal glomeruli (Figs. 2, 3, and 4). Although no

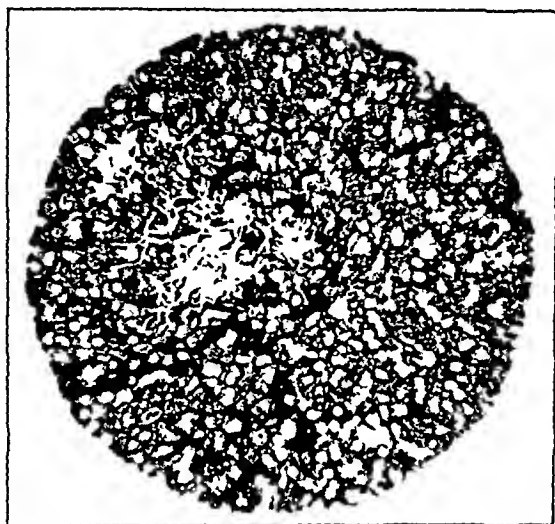


Fig. 3.—Section of human spleen. High dry lens. Approximately 440 diameters magnification. Note clump of bacteria. (Lillie's stain.)



Fig. 4.—Section of human kidney. Oil immersion view of glomerulus. Approximately 1080 diameters magnification. Note two anthrax bacilli partly superimposed on each other, in the right lower field. (Lillie's stain.)

serial sections were made, it was obvious from the study of our material, that death was not caused by the mechanical blockade of the capillary bed, as maintained by some pathologists. Even in the guinea pig, where the of organisms found in the tissues was enormous, a great many 'good' , and even those that showed many organisms, were only pe

## COMMENT

Although within forty eight hours after admission to the hospital the patient received 315 cc of serum intravenously, in small repeated doses, and 30 cc locally around the lesion, the course of the disease as measured by the edema and general intoxication was progressively for the worse. It is of interest to note that the spread of the edema occurred very soon after the local injections of serum, and though it was interpreted at first as a local reaction similar to that reported by other observers,<sup>1, 2</sup> it was later found at autopsy to be the result of a direct spread of the anthrax bacilli along the tissue spaces. It is our impression that the local injection of serum by mechanically separating the tissue spaces facilitated, rather than hindered, the spread of the organisms. Since a pronounced effect upon the local edema can be obtained by the intravenous injection of large doses of serum as evidenced by our other cases, we feel that the injection of serum around the lesion is of no particular advantage, and that it may actually do harm. Although in the light of our later experience the amount of serum given to this patient was small, it is well to remember that he was seen early in the disease, and that recoveries have been reported in similar or worse cases through the use of even smaller doses of serum. It is remarkable that a large dose of serum given six hours after the blood was found to be sterile did not prevent the death of the patient ten hours later, when the organisms were recovered from his heart and spleen.

CASE 2—T S, a white male, aged twenty four, was seen on the morning of May 19, 1933, when he stated that on the previous day, he had scratched his hand while cleaning a brush in the carding room. The left middle finger showed three fine linear scratches about 0.5 cm long on the dorsal surface above the middle joint. Adjacent to the middle scratch, there was a small, red, flat painless papule, hard to the touch. Temperature and pulse were

TABLE II

## CASE 2

DATE	TIME	SERUM	ROUTE	REACTION	WBC'S	REMARKS
5/20/33	1 00 A M	300 cc	Intraven	Slight chill 102° fever		Temperature normal in A M WBC, 10,700
	4 00 P M	200 cc	Intraven	Slight chill T 100.4		Polys 80 per cent Blood culture sterile
5/21/33	8 00 P M	50 cc	Intramusc	Local pain		Patient feels well Three painless swollen glands present
5/22/33	9 00 P M	50 cc	Intramusc	Local pain		WBC, 7,500 Polys, 56 per cent
5/23/33	4 00 P M	100 cc	Intraven	T 100		Smear positive for anthrax
5/31/33	9 30 P M	40 cc	Intramusc			Mild serum sickness since 5/25 Lesion had en- larged with a purple blue bleb at its margin
6/ 1/33	10 00 A M	100 cc	Intraven	T 99.6		Serum sickness aggravated Bleb spreading Smear positive for anthrax
6/ 2/33	10 00 P M				0.6 gm	
6/ 3/33	10 00 A M	100 cc	Intraven	T 99.4	0.9 gm	Mild serum sickness Le- sion enlarging
6/ 4/33	10 00 A M				0.9 gm	Blood culture taken two days later sterile
6/10/33						Horse serum, I 100, intra- cut Negative Horse se- rum 1 10, intracut Slightly positive

normal. Six hours later he complained of headache and malaise. Temperature was 102° and pulse 100. The papule, still hard and red, had enlarged to the size of a dime. There was slight, nonpitting edema. The left epitrochlear and axillary nodes were palpable, the latter being painful. On incision, there exuded from the lesion a few drops of thick, yellow fluid from which anthrax bacilli were recovered.

The course in the hospital is outlined in Table II.

Notwithstanding the evidence of serum sickness, antianthrax serum, reinforced by neoarsphenamine, was given on June 1 because of the marked spread of the lesion, the appearance of a bluish red blister at its edge, the persistent adenopathy, and the continued presence of anthrax bacilli. No untoward reaction resulted. On June 7, 1933, the skin of the blister and the eschar were removed, leaving a shallow ulcer which healed completely in about two weeks.

CASE 3.—W. S., a white boy, ten years of age, was admitted to the Chester Hospital on the night of Sept. 10, 1933, with a diagnosis of anthrax of the face. The temperature was 102.4°, pulse 110. The lesion was a typical carbuncle in a rather advanced stage (third day). The right submaxillary triangle was swollen, inflamed and tender, with a red streak running across it to the sternoclavicular joint. No glands were felt. When we saw the patient in the morning of the eleventh, the edema had increased a little. Serum was given according to Table III.

On Sept. 26, 1933, the eschar was removed leaving a small punched out ulcer which healed in ten days leaving a small flat scar. On October 14, intracutaneous testing with horse serum

TABLE III

CASE 3

DATE	TIME	SERUM	ROUTE	REACTION	NEOARSPH.	REMARKS
9/11/33	2:30 P.M.	450 c.c.	Intraven.	104° fever Slight chill		Temperature down to 99.2° in 6 hours. Blood culture sterile. Urine showed a heavy cloud of albumin
	11:45 P.M.	500 c.c.	Intraven.	104.4° fever Slight chill	0.45 gm.	Neck less swollen. Not tender
9/12/33	1:00 P.M.	200 c.c.	Intraven.		0.6 gm.	Swelling markedly decreased. Two small cervical glands on the right. W.B.C., 13,500. Polys, 86 per cent
9/13/33	11:00 A.M.	100 c.c.	Intraven.		0.6 gm.	Temperature normal. Hard swelling at angle of jaw due to lymph node. Lesion enlarging. Urine shows trace of albumin
9/14/33	5:00 P.M.	50 c.c.	Intramusc.	Local pain		Gland at angle of jaw size of walnut. Temperature normal
9/20/33	3:30 P.M.	50 c.c.	Intramusc.	Local pain		On 9/16/33, lesion negative for anthrax bacilli. Large node at angle of jaw hard, not tender. Few cervical glands on the right. Lesion dry with black eschar. W.B.C., 7,600. Polys, 63 per cent
9/29/33	Discharged from the hospital					Horse serum, 1:100, intracut. Test negative



Fig 5—Case 2 Lesion as it appeared on the third day of illness. Note the ulcer and the swelling of the joint.

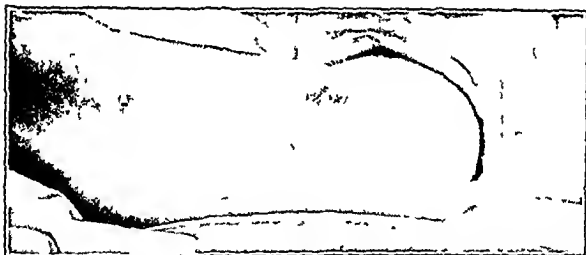


Fig 6—Case 4 Lesion as it appeared on day of admission to the hospital. Note the ring of vesicles around the ulcer and the marked edema of the forearm.



Fig 7—Case 4 Lesion as it appeared on day of discharge from the hospital after treatment. Note the epithelial islets at site of former carbuncle and the disappearance of the edema.

(1-10) was plus-minus. The patient had never had serum sickness in spite of the large repeated doses of antianthrax serum.

CASE 4.—W. D., a white male, eighteen years of age, was well until Oct. 6, 1933, when he scratched his left forearm while at work in a Philadelphia plant, which obtained its raw material (hair bobbins) from the Delaware County mill. Two days later he noted a small, painless, red pimple at the site of the untreated scratch. This was self-treated until the twelfth, when he was referred to us for diagnosis. Examination revealed a well-developed lesion with a central black eschar and four surrounding vesicles on the external surface of the left forearm below the elbow. The upper half of the forearm was tensely swollen and somewhat hot. The skin was slightly red. The left epitrochlear gland appeared as a visible tumor the size of a large walnut. Two axillary nodes were large and painful. There was a negative history for allergy, and the patient had never received any horse serum before. The patient was admitted to Chester Hospital on Oct. 12, 1933, at 1:45 p.m.

TABLE IV

## CASE 4

DATE	TIME	SERUM	ROUTE	REACTION	NEOARSPIH.	REMARKS
10/12/33	8:00 P.M.	1000 c.c.	Intraven.	In the middle of the injection patient got hot and forehead itched. Toward the end, a few small hives and pruritus appeared, lasting 5 minutes. Slight chill. T. 99.8°		Throughout illness he had a very slight febrile response. Urine showed a cloud of albumin. W.B.C., 8,350. Polys, 81 per cent. Blood culture sterile
10/13/33					0.9 gm.	Few hives on face. Edema decreased
10/14/33	3:00 P.M.	100 c.c.	Intramusc.	T. 100° Local pain	0.9 gm.	Epitrochlear and axillary glands larger
10/15/33					0.9 gm.	
10/18/33	9:00 P.M.	100 c.c.	Intramusc.	Few hives and pruritus		Vesicles around the lesion dry. Smear positive for anthrax bacilli. Diffuse adenopathy
10/23/33						Eschar removed. Shallow ulcer. Healed in 2 weeks
10/28/33						Discharged from hospital. Diffuse adenopathy still present. Horse serum (1-10) intraent. Test slightly positive

A week after discharge the generalized adenopathy had subsided, except for the left epitrochlear gland which was still very prominent. It decreased in size very gradually and was both palpable and visible for more than six weeks. We were tempted to remove it to determine whether it harbored live anthrax bacilli, but we feared that surgical manipulation might activate this possible latent focus.

CASE 5—A K, a white male forty years of age, was working in the carding room in the noon shift on Nov. 1 1933 when he scratched the right side of the neck with a drum wire about 7 00 P M. The next day when he reported to work at 2 00 P M, a boil was noted at the site of the scratch, and he was referred to us for treatment. There was a "carbuncle" over the mesial border of the right sternomastoid muscle with an area of painless edema three inches wide. Temperature and pulse were normal (Table V).

On November 13 the lesion began to show involuntarily changes. The eschar was removed on the fifteenth and the patient was discharged five days later. The ulcer was completely healed in about ten days.



Fig. 8—Case 5. Taken thirty-six hours after treatment was begun. All edema disappeared. Note collar of tense vesicles around central ulcer.

CASE 6—H G, a white boy twelve years of age, lived in the village right at back of the curling room. He was seen on the evening of Dec. 12 1933, when he gave the following history. On December 7 he noted a pimple on the right upper cheek. Two days later it looked the same, itched a little, but there was no pain or swelling. On the tenth the sister scratched him above the pimple, drawing some blood. The pimple turned black while he was at school. When he returned home, his mother noted that the entire right cheek was swollen. He was feverish and complained of headache and pain in the upper right side of the neck. On examination temperature was 100.4° and pulse 110. The right cheek was swollen and showed a carbuncle in its center, with a deep scratch 1½ cm. above it, but bearing no relation to it. The lesion had a black dry center. All the cervical glands were enlarged on the right. The boy had received diphtheria toxin antitoxin in 1928.

On December 25 the patient was well, the ulcer had completely healed and the adenopathy disappeared three days after discharge from the hospital.

TABLE V

## CASE 5

DATE	TIME	SERUM	ROUTE	REACTION	NEOARSPH.	REMARKS
11/2/33	7:00 P.M.	750 c.c.	Intraven.	Severe chill T. 100° Vomiting	0.9 gm.	Blood culture sterile
11/3/33					0.9 gm.	Swelling less marked. Ulcer enlarged. On its upper edge appeared a semilunar ring of tense dark vesicles
11/4/33						W.B.C., 9,600. Edema disappeared. Ulcer spreading
11/6/33	3:30 P.M.	100 c.c.	Intramusc.	T. 100° Local pain		Fluid from a blister showed a sparsity of cells, half of which were polys
11/8/33	9:00 P.M.	100 c.c.	Intramusc.	T. 100°		Occipital headache. Nuchal pain. No skin reaction. Joints and reflexes normal. Serum sickness? Meningitis? Smear negative for anthrax
11/9/33						Pruritus. Macular rash lasting 48 hours. Ulcer dry. Smear negative for anthrax

TABLE VI

## CASE 6

DATE	TIME	SERUM	ROUTE	REACTION	REMARKS
12/12/33	9:30 P.M.	750 c.c.	Intraven.	Slight chill T. 100.2°	Blood culture sterile
12/13/33	3:30 P.M.	250 c.c.	Intraven.	Slight chill T. 102°	Swelling unchanged. Lesion enlarging. Glands are the same. W.B.C., 11,600. Polys, 85 per cent. Urine shows cloud of albumin
12/14/33					Temperature normal. Faint erythema. Swelling decreased. Lesion unchanged
12/16/33	9:00 P.M.	50 c.c.	Intramusc.	Slight pain	Large blister, the size of a nickel, containing clear fluid appeared at the lower edge of the wound
12/17/33					Mild serum sickness present. Swelling of the cheek still present. Blister turning black. Right upper neck swollen, due to enlarged glands
12/21/33					Severe serum sickness. Hives. Erythema. Chemosis and edema of the lids. T. 103°. Reaction lasted 3 days. Lesion dry, eschar removed. Smears taken on 12/19 and 12/23 negative for anthrax bacilli



Fig 9—Case 6 Taken before treatment on night of admission to hospital



Fig 10—Case 6 Taken four days later. Note the superficial blister at the lower edge of the ulcer. This contained clear fluid



CASE 7.—On Feb. 2, 1934, we saw a white man, S. P., aged twenty-four, who worked in the carding room of the mill. He had a lesion in the middle of the right calf the size of a quarter, with a dry black center, and a ring of tense flat red vesicles around it. The contour of the leg was obliterated by a tense and painfully tender swelling. The temperature was 100.4°. He had had tetanus antitoxin in 1930. He had apparently scratched his leg while at work. Smear and culture from the lesion were positive for anthrax bacilli.

On Feb. 2, 1934, at 6:00 P.M. the patient received 300 c.c. of serum intravenously, with no reaction. Twenty-four hours later the swelling had disappeared entirely. The blood culture was sterile; W.B.C. 8,000; polys 60 per cent. The lesion ran its natural course of peripheral vesiculation, drying of the vesicles, shrinking of the eschar and its separation, which occurred on the tenth. On the seventh the patient developed marked serum sickness which lasted four days. He was discharged from the hospital on February thirteenth, and he returned to work on March 5, 1934.

CASE 8.—A. K., a white female, forty-three years of age, had quit work in the weaving department of the mill, where she carried hair-bobbins, on Feb. 3, 1934. At a site of a scratch on the face, she developed a sore and a swelling which she treated herself without success. On February 7 we saw her at her home. She was found in the kitchen, her face bandaged in rags, and the examination was made under threat of calling the police. The left face was badly swollen, from the lower orbital ridge to the cervical crease below the jaw. In the center of the cheek, there was a very ugly black ulcer, larger than a twenty-five cent piece. It was surrounded by a ring of dark vesicles and a zone of erythema. The sore was painless. The gland at the angle of the jaw, the sublingual and the submaxillary nodes on the left side were swollen, hard, tender, and easily outlined. There was no cervical adenopathy. On admission to the Chester Hospital, the temperature was 99.4° and pulse 90.

TABLE VII

## CASE 8

DATE	TIME	SERUM	ROUTE	REACTION	REMARKS
2/ 7/34	7:00 P.M.	300 c.c.	Intraven.	Slight chill Fever 103° Pulse 130	Malaise. Lesion larger, blisters oozing freely. Edema spreading around the jaw. Blood culture sterile. W.B.C., 8,500. Polys, 68 per cent
2/ 8/34	11:00 A.M.	300 c.c.	Intraven.	Slight chill: Fever 103.2° Pulse 116	Temperature normal overnight. Edema spreading
2/ 9/34	10:30 A.M.	500 c.c.	Intraven.	No reaction	Temperature before injection 102°. Edema more marked. Lower lid swollen. Nasal outline distorted. Glands tender
2/10/34					Within 4 hours of the last injection edema decreased considerably, temperature and pulse returned to normal. Glands remained the same. Ugly black ulcer, larger than a half dollar. Peripheral blisters open, leaving a bright red edge around the lesion. Smear shows <i>Staphylococcus albus</i>
2/11/34	3:30 P.M.	100 c.c.	Intramusc.	No reaction	Edema completely gone. Glands decreasing in size, though still palpable. Nonpainful

On the thirteenth, the temperature went up to 100°, and skin manifestations of mild serum sickness appeared. The lesion was smaller, and the eschar was softening. Smear and culture still showed an occasional anthrax bacillus. The hives and erythema lasted thirty-six hours.

On the sixteenth, the eschar was removed leaving an infected ulcer. Two days later, the patient was discharged from the hospital. She had been very uncooperative during her entire sickness, and a religious psychosis which was present before she contracted anthrax, made treatment very difficult. The glands had completely disappeared and the wound



Fig 11—Case 8 Taken on day of admission to the hospital. Note the marked edema of the entire left cheek and jaw.

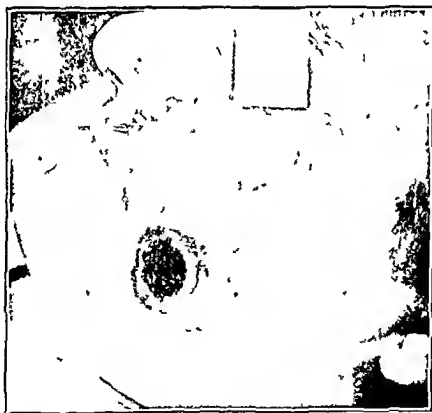


Fig 12—Case 8 Taken after treatment. Note the shrunken eschar with dry edge where vesicles were present. Observe total disappearance of edema.

was in the process of healing when she left the hospital on the eighteenth. We never saw her thereafter, but the husband reported that she was perfectly well and that her wound had healed in about two weeks.

CASE 9.—A. W., a white male, forty-eight years old, working in the card room, on Jan. 22, 1935, struck the right side of his face above the zygoma, with the loose end of a steel band, when he attempted to cut it, to open a compressed bale of hair. The scratch was immediately and thoroughly cauterized with pure phenol and painted with iodine. He continued to work, the wound being tightly covered with a sterile dressing. On Jan. 26, 1935, while at home in Camden, N. J., the face became swollen and a sore that turned black in twenty-four hours appeared at the site of the scratch. The patient did not report to my office until January 30, when the typical late lesion (eschar and vesicles) of cutaneous anthrax, plus extensive edema of the right side of the face with obliteration of the submaxillary space, were found. The submaxillary node was large, hard, and very tender, but the upper cervical nodes on the right were pea-like. He was admitted to the Chester Hospital with a temperature of 99.6° and a pulse of 80.

On February 4, anthrax bacilli were recovered from the lesion. But two days later, smear and culture proved to be negative. On the fifth, the eschar had spread to the peripheral vesicles and a large swelling, the size of a small orange, was found behind the angle of the jaw. It was due to a lymph node. Temperature 99.4°. On the sixth, temperature 101°,



Fig. 13.—Case 3. Taken on day of admission to hospital. Note extensive edema present.

pruritus and crops of hives and erythema appeared, lasting forty-eight hours. The febrile reaction lasted for over a week. Although there was a diffuse, small adenopathy due to serum sickness, the right upper neck was tensely swollen because of large nodes. On the ninth, the eschar was removed, leaving an ulcer, the size of a nickel, with an infected base. On the fifteenth, the temperature dropped from 102° to normal. The nodes at angle and below the jaw were the size of walnuts, and firm to the touch. A pebble-like node was felt in front of the auditory meatus. All other nodes disappeared. Patient was sent home the next day. The submaxillary nodes decreased in size very slowly and were palpable for over two weeks. The ulcer gradually healed, leaving a very small scar.

CASE 10.—A. S., a white male, nineteen years old, who worked in the basement of the carding room (no other case had occurred there), was seen in my office, on Feb. 7, 1935. A small dry, yellowish, pea-like papule, located in the center of a very bright erythematous area, the size of a quarter, was found on the right upper neck. There was neither edema nor cervical adenopathy. He complained of malaise and anorexia. The temperature was 100 and the pulse was 96. A smear obtained by rubbing an applicator, moistened with normal saline, against the papule, confirmed the diagnosis of anthrax.

On February 11, pruritus and urticaria appeared. Temperature was normal. On the fourteenth, there was marked serum sickness. Temperature rose to 102.104°, lasting four days. Patient also complained of migratory joint pains. On the eighteenth, patient was normal. Most of the eschar was removed leaving an infected ulcer. There was no adenopathy. Patient was discharged on the twenty-second. Ulcer healed in two weeks.

TABLE VIII

## CASE 9

DATE	TIME	SERUM	ROUTE	REACTION	REMARKS
1/30/35	4 45 P M	500 cc	Intraven	None	Eye and skin tests to horse serum, neg. blood culture, neg. Smear and culture from lesion, positive for anthrax
1/31/35	10 15 A M	500 cc	Intraven	None	W B C, 8,750 Polys, 65 per cent. Face more edematous, with partial closure of eye. Swelling behind and below ear. Tenderness over submaxillary node disappeared 6 hr after first injection, size unchanged. Lesion spreading.
1/31/35	8 00 P M				Vesicles open. Swelling of face almost entirely gone. Lymph nodes showed no change.

TABLE IX

## CASE 10

DATE	TIME	SERUM	ROUTE	REACTION	REMARKS
2/ 7/35	3 30 P M	200 cc	Intraven	None	Eye and skin tests, neg. for horse serum.
2/ 8/35	10 30 A M	300 cc	Intraven	Slight chill T 103°	9 00 A M T 101°. Lesion larger. Erythematous base is gone, but the center of the papule is turning black, with peripheral ring of fine, first vesicles. Large area of tender edema has developed around the lesion. Upper anterior cervical nodes palpable.
2/ 8/35	6 00 P M				Tenderness over edematous area gone. Swelling decreasing.
2/10/35					Edema disappeared entirely. Smear and culture, negative for anthrax.
2/12/35					W B C, 9,500 Polys 84 per cent.

## COMMENT

The last two crises occurred within two weeks of each other, in different parts of the same building, a year after Case 8 had developed. During this anthrax free period, no changes were made in the raw material employed, or in the processing to which it was subjected. The question arose, as to whether we were dealing with a recurrence of the old infection, or whether a new shipment of hair which had just arrived at the mill was responsible for the fresh outbreak. Tests similar to those described in the early part of this report, were made on samples of hair, obtained (1) from the center of a new bale, (2) from the surface of a new bale, (3) from a bale already opened and in the process of being carded, and (4) from sweepings of the storeroom floor, which contained dust as well as hair. The results were as follows: (1) Positive for anthrax. Three out of six guinea pigs died. (2) Negative for anthrax. (3) Negative for anthrax. (4) Positive for anthrax. Two out of six guinea pigs died.

These tests would indicate that in all probability the new outbreak of anthrax was caused by a recently imported lot of hair, which was contaminated.

It is of interest to note that in Case 9, thorough cauterization of the wound with pure phenol, immediately after the workingman was injured, did not kill the anthrax bacilli, although it may account for the rather long incubation period present. In Case 10 (which was seen very early, in the papule stage), the administration of 200 c.c. of serum intravenously did not prevent the occurrence of local edema and regional adenopathy. However, the latter was controlled by further administration of 300 c.c. of serum, indicating that a certain optimum dose is required in each individual case.

#### DISCUSSION

Antianthrax serum (human), manufactured by the Mulford Laboratories, Sharp and Dohme, was used in the treatment of all these cases. After the unfortunate experience with Case 1, we discontinued the use of serum locally, and depended entirely upon the intravenous route of administration. Occasionally, late in the disease, when slow absorption of the serum was desired, the intramuscular route was employed. Large quantities of serum were given slowly by the gravity method. The average total dose given in the recovered cases was 943.3 c.c. The smallest total dose was 300 c.c., while the largest was 1,350 c.c. of serum. The smallest single intravenous injection was 100 c.c., while the largest single injection of serum by vein was 1,000 c.c. Single doses of 400 to 500 c.c. of serum were used most frequently in our cases.

The anthrax infection, although localized to the skin, produces a systemic intoxication as evidenced by fever, rapid pulse, and malaise, which disappear under specific treatment. Our experience with these cases convinced us that an optimum dose of serum was necessary for each case, and that once that dose was given, definite results were to be expected. The temperature and pulse dropped sharply, while the edema showed a spectacular decrease and total disappearance within a short period of time. This local effect of the serum upon the edema must depend entirely upon the neutralization of some bacterial secreted factor, because neither the organisms themselves, nor the original adenopathy, showed any change after its administration. Thus anthrax bacilli were recovered from the lesions in Cases 2, 4, 8, and 9, very late in the disease, when the patients seemed to be well on the road to recovery. This persistence of the organisms and the marked regional adenopathy, which some observers consider as a dangerous possible source of reinfection<sup>4</sup> made us continue our specific treatment late in the disease. This appears, perhaps, unnecessary, in view of the fact that the glands remained enlarged for days and even weeks after the lesion was completely healed; yet the patients felt perfectly well, and made an uneventful recovery. In Case 9, enough serum was given to control the edema; the local lesion and the adenopathy received no further treatment, yet they underwent complete involution with restoration to normal. We are unable to state whether the glandular enlargement is due to actual invasion of the nodes by the anthrax bacilli, or whether they merely represent an inflammatory reaction to the products of the bacilli, which are themselves localized in the carbuncle. Serum does eliminate the pain which is sometimes present in the enlarged glands.

The course and rate of development of the carbuncle, however, appear to be unaffected by the administration of serum

The antianthrax serum used in these cases did not produce any severe reaction, and the serum sickness which it induced in some of our patients was rather mild. The previous administration of horse serum somewhat aggravated the latter. This absence of severe reaction is particularly noteworthy in view of the tremendous doses of horse serum used intravenously in our cases. Because of this finding we administered the serum, in the presence of serum sickness without running into any difficulties.

The use of neoarsphenamine in large doses, which has been advocated by several European workers<sup>2</sup> was in our experience of no value. When given in addition to the serum it did not appear to alter or shorten the course of the disease, and its use was discontinued in our last five patients.

The leucocyte response in our patients was not very significant. It was normal in most of them with leucopenia and slight leucocytosis in a few. After administration of serum no noteworthy leucocyte response occurred, hence we feel that the *modus operandi* of specific serotherapy does not depend upon the mobilization of the white blood cells as claimed by some.<sup>6</sup>

It is interesting to speculate on the nature of the processes of recovery in those infected with anthrax and on the possible immunity conferred by an attack of this disease. Facts bearing upon this phase of the problem, as well as results obtained during attempts at active immunization of human beings by means of intracutaneous and subcutaneous administration of an anthrax antigen will be reported in a separate communication.

It is obvious that treatment of the wounds, even when done early, is of no value in the prevention of anthrax. The hope for prophylaxis lies in the enforcement of strict laws governing the importation of hair from areas where anthrax is endemic, the possible disinfection of all hair at ports of entry under government control as it is done in England, and the development of suitable means of active immunization against this disease.

#### SUMMARY

1 Ten cases of anthrax are here reported. Two patients were children, and one case was a woman.

2 Seven of the patients contracted anthrax while working in a mill, which uses imported goat hair for its raw material. The two children lived in the village surrounding the mill immediately behind the carding room which was the center of infection. The other case occurred in a distant plant where the infection was carried from the local mill, via shipping cases or hair bobbins.

3 All patients but the first one, recovered.

4 Antianthrax serum was used intravenously in large doses in the patients who recovered.

5 Injection of the serum locally, around the anthrax lesion, was discontinued after the first case, because of the possible dangerous spread of the infection through the tissue spaces. This may be brought about by the mechanical separation of the tissues.

6. Neoarsphenamine was not found to be of any value in the few cases where it was given in addition to serum.

7. Studies to determine the source of infection, are given. Tests indicate that a rather virulent strain was responsible for the cases of anthrax reported.

8. Autopsy findings are given on the one death of our series.

I wish to express my gratitude to Drs. John Reichel and J. E. Schneider, of Mulford Biological Laboratories, Sharp and Dohme, Glenolden, Pa., for their kind interest and valuable advice given during this study.

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314 EAST BROAD STREET

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### THE INCIDENCE OF NONDIABETIC GLYCOSURIA\*

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PHYSICIANS generally recognize that a small percentage of persons showing glucose in successive urine specimens do not have diabetes mellitus, that the condition is renal hereditary glycosuria.<sup>1</sup> Many are cognizant of the existence of another type, usually intermittent, of glycosuria, best described as nondiabetic glycosuria.<sup>2</sup> Careful search of the literature disclosed a single statistical investigation of the subject: Joslin<sup>3</sup> states that one-sixth of the persons referred to him on account of glycosuria did not have diabetes. Several physiologic experiments<sup>4</sup> point to the existence of this type of glycosuria, which none the less seems to have been generally overlooked in clinical literature. Isolated case reports<sup>5</sup> of persons with benign glycosuria, some of them mistakenly treated for diabetes for years,<sup>6</sup> appear infrequently. Several classifications<sup>7</sup> of the glycosurias have been proposed; all seem unsatisfactory for reasons which need not be discussed here.<sup>8</sup> The most satisfactory classification of the glycosurias would seem as follows: diabetic and nondiabetic.

It is my purpose to present a statistical analysis of 238 persons referred to the Diabetic Division of the Aaron Waldheim Health Clinic during the last six years with a tentative diagnosis of diabetes or on account of glycosuria

\*From the Diabetic Division of the Aaron Waldheim Health Clinic and the Jewish Hospital.

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demonstrated in one or several urine specimens. Thirty-two (13 per cent) had nondiabetic glycosuria. Table I based on the glucose tolerance curve<sup>9</sup> summarizes these cases. Repeated tolerance tests were used as a check on the diagnosis. In no case diagnosed as nondiabetic glycosuria has it been necessary later to make a diagnosis of diabetic glycosuria, occasional patients at first considered to have diabetic glycosuria have later been considered nondiabetic.

#### CASE REPORT

A single case report atypical in many respects yet illustrative of the fact that hyperglycemia during the tolerance test does not of necessity mean diabetes, is given. This thirty year old white female first came to the clinic in 1928 on account of a duodenal ulcer which has since been quiescent under medical management. During hospitalization in 1929 a routine glucose tolerance test was done. In May 1930 the patient first reported to the diabetic division because of pruritis vulvae; there have been no other complaints suggestive of diabetes. The tolerance curves are shown in Table I.

TABLE I  
TRUE BLOOD SUGAR IN MG PER CENT BEFORE AND AFTER 100 GRAMS GLUCOSE

DATE	FASTING	1	2	3	4 HOURS	BODY WEIGHT POUNDS
May, 1929	112	209	180	126		130
May, 1930	117	211		58		152
June, 1931	76	270		177		121
Oct., 1931	113	194	116	135	77	130
June, 1934	120	212	219	167		140
Dec., 1934	116	212	120	90		132
Oct., 1935	116	194	123	100	80	132

Of 62 urinalyses during this interval 28 showed sugar in varying amounts, 34 no sugar. Glycosuria occurred without regard to blood sugar level. The absolute dissimilarity of the curves should be noted. Had this patient been seen for the first time in June, 1931 or June, 1934, a diagnosis of diabetes would have been made—to be shown wrong in December, 1934. This unusual case has been presented instead of one more typical of nondiabetic glycosuria because it illustrates well a type of patient in whom I think antidiabetic therapy is not indicated.

#### DISCUSSION

To discuss the mechanism of glycosuria would carry us far afield and lead to no useful result.<sup>10</sup> I am of the opinion that there is no relationship between the level of sugar in the blood and its appearance in the urine. My interpretation of the tolerance curve is based upon the relationship existing between a normal or nearly normal<sup>11</sup> fasting blood sugar level and the blood sugar level three hours after the ingestion of 100 gm of glucose. If the latter is below 100 mg per cent a diagnosis of nondiabetic glycosuria (should sugar appear in the urine) may unhesitatingly be made, if above 150 mg per cent, a diagnosis of diabetes is almost always certain (regardless of the appearance of sugar in the urine), if the three hour blood sugar level is between 100 and



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METROPOLITAN BUILDING

## A CHEMICAL STUDY OF THE ALUM DIPHTHERIA TOXOID PRECIPITATE\*

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HAVENS and Wells<sup>1</sup> showed in 1933 that diphtheria toxoid precipitated by an alum solution the precipitate washed once with saline and then suspended in fresh saline, gave a product which in one inoculation immunized practically all of the children so treated. Prior to this Park and Schroeder<sup>2</sup> obtained results in guinea pigs and children superior to those with ordinary toxoid, by using a toxoid containing 0.2 per cent alum, partially precipitated and not purified.

A detailed study of the conditions for preparing this diphtheria toxoid alum precipitate as developed in this laboratory, and some of the properties of the precipitate are presented in this paper.

The diphtheria toxoid used was made from bacto veal<sup>3</sup> broth and fresh veal broth by Dr. O. R. Povitzky, to whom we wish to express our thanks. The different lots of this material averaging about 150 litres each were given a preliminary test to determine the conditions for precipitation that would yield the best obtainable product since they showed such markedly diverse reactions upon the addition of alum. This method takes into account the factors of temperature, concentration of alum, and inability to precipitate some lots of the toxoid by the addition of alum alone until sodium chloride has been added. A 10 per cent solution of potassium aluminum sulphate,  $K_2Al_2(SO_4)_4 \cdot 24H_2O$ , analytical reagent quality, was used.

A series of 108 test tubes, each having a capacity of about 8 c.c., was set up in 9 racks, each rack containing 12 tubes in 2 rows. To each tube, 5 c.c. of the toxoid being tested were added. The racks were segregated into 3 sets. One set was heated in a water bath at 60° C. for 15 minutes and varying quantities of the 10 per cent alum solution were added to each row of tubes to give a final concentration of 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 per cent alum. The tubes were shaken and heated for an additional 15 minute period. Solid sodium chloride was then added so that for a given concentration of alum in toxoid there would be 1, 2, 3, 4, and 5 per cent salt, and one as a control without salt. The tubes were shaken to dissolve the salt and bring down the precipitate. They were left at room temperature and read twenty-four hours later.

The other two sets of tubes were treated in the same way with the exception of the temperature factor. They were heated in a water bath to 40° C. prior to the addition of the alum and salt. One set was put in the incubator at 37.5° C. until the following morning, while the other was left at room

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temperature. The amounts and appearances (granular, flocculent, etc.) of the precipitates and the appearances of the supernatants (clear, cloudy) were noted after twenty-four hours.

Two tables are presented giving the results of such preliminary tests, Table I for a toxoid prepared with bacto-veal broth, and Table II for a toxoid prepared with fresh veal broth. The "bacto-veal" toxoid gave the most satisfactory precipitates only on the addition of 4 per cent or 5 per cent sodium chloride and with 1 per cent or 1.5 per cent alum. Only in these cases were the supernatants clear. With the "fresh veal" toxoid (Table II) on the other hand, the most satisfactory precipitates were obtained with 2 per cent or 2.5 per cent alum and no added salt. In neither table were the 60° C. precipitates as satisfactory as those obtained at the lower temperatures.

These two tables are typical of the results obtained with the various toxoid preparations.

*Final Test.*—From the tables of the initial test, 4 or 5 tubes were chosen showing the greatest amount of precipitate, finely divided rather than granular, having the clearest supernatant fluid. For the toxoids shown in Tables I and II, the preparations marked with an asterisk were selected. Using the same concentrations of alum, and salt or no salt as indicated, 100 c.c. portions of the toxoid were precipitated under the same conditions to yield sufficient material for quantitative tests. The suspensions were allowed to settle for four hours, the supernatant fluids drawn off and the precipitates washed with saline equivalent to the amount of fluid removed. The following morning the washings were drawn off and enough saline added to make the suspensions up to the original volumes of toxoid. The suspensions were dissolved by the addition of 1 per cent solid sodium citrate and warming in water-bath at 40° C., and tested for flocculating units (Lf),<sup>4</sup> total nitrogen by the Kjeldahl method, and aluminum content.

The method for the determination of aluminum was adapted for use on diphtheria toxoid-alum precipitate by Clifford George Pope<sup>5</sup> and is most reliable where quantities of material are tested containing 0.5 to 1.0 mg. of aluminum. The toxoid-alum suspension was measured out, dissolved in 5 per cent borax solution, and the aluminum precipitated quantitatively with 8-hydroxy quinoline. The aluminum-quinoline precipitate was dissolved in hot hydrochloric acid (1:3) and the contained aluminum estimated by titration with excess standard potassium bromate-bromide and back titrated with standard sodium thiosulphate solution as in the Koppeschaar method.

The data given in Table III refer to the toxoid preparations for which the results of the preliminary tests were presented in Tables I and II.

The flocculation determinations were carried out in Dr. Olga R. Povitzky's division by Miss Minnie Eisner to whom thanks are due. So far as the character of the precipitate is concerned, a finely dispersed material is most desirable so that a uniform mixture for injection can readily be obtained. For the toxoids shown in Table III, the "veal" toxoid precipitate was well dispersed and slowly settling, while the "bacto-veal" toxoid precipitate was coarser in character and settled more rapidly. This difference was not always

TABLE I

COMPARATIVE STUDY OF FORMATION OF DIPHTHERIA TOXOID ALUM PRECIPITATE WITH VARYING QUANTITIES OF ALUM AND SODIUM CHLORIDE AT DIFFERENT TEMPERATURES (BACTO VEAL)

ALUM	CONTROL			1% NaCl			2% NaCl			3% NaCl			4% NaCl			5% NaCl			60° HEAT
	1% C	ROOM	60° HEAT	1% C	ROOM	60° HEAT	1% C	ROOM	60° HEAT	1% C	ROOM	60° HEAT	1% C	ROOM	60° HEAT	1% C	ROOM	60° HEAT	
Supernatant	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.5% Precipitate	++	+	-	++	+	-	++	+	-	++	+	-	++	+	-	++	+	-	++
1.0% Precipitate	++	+	-	++	+	-	++	+	-	++	+	-	++	+	-	++	+	-	++
1.5% Precipitate	++	+	++	++	+	++	++	+	++	++	+	++	++	+	++	++	+	++	++
2.0% Precipitate	-	-	++	-	-	++	-	-	++	-	-	++	-	-	++	-	-	++	++
2.5% Precipitate	-	-	++	-	-	++	-	-	++	-	-	++	-	-	++	-	-	++	++
3.0% Precipitate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Supernatant	+++ (maximum cloudiness)			→			→			→			→			→			-
Precipitate	+++ (maximum)			→			→			→			→			→			-
																			-

(clear)  
(none)

TABLE II

COMPARATIVE STUDY OF FORMATION OF DIPHTHERIA TOXOID ALUM PRECIPITATE WITH VARYING QUANTITIES OF ALUM AND SODIUM CHLORIDE AT DIFFERENT TEMPERATURES (VEAL)

ALUM	CONTROL			1% NaCl			2% NaCl			3% NaCl			4% NaCl			5% NaCl		
	INC.	ROOM	60° HEAT	INC.	ROOM	60° HEAT	INC.	ROOM	60° HEAT	INC.	ROOM	60° HEAT	INC.	ROOM	60° HEAT	INC.	ROOM	60° HEAT
Supernatant	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.5% Precipitate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Supernatant	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1.0% Precipitate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Supernatant	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1.5% Precipitate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Supernatant	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2.0% Precipitate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Supernatant	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2.5% Precipitate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Supernatant	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3.0% Precipitate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

↑ (maximum cloudiness)  
 ↑ (clear)  
 (none)

found; that is to say the "veal" toxoid precipitate was not always more satisfactory so far as dispersion and settling were concerned. The individual preparations, whether from "veal" or "bacto-veal," varied without any apparent regularity in these properties

The analytical data and calculations shown in Table III require little elaboration. The Lf per mg N serves as an index for the satisfactory conversion of the diphtheria toxoid into the alum-toxoid precipitate, the greater the Lf and the smaller the N content per c c, presumably the more satisfactory

TABLE III  
FINAL TEST

SOLUTION TESTED	LF/C C	MG N/C C	LF/MG N	MG AL/C C	MG AL/MG N	LF/MG AL
Diphtheria Toxoid Preparation No 41 (Bacto Veal)						
Original toxoid (bacto veal)	10.3	4.41	2.3	0.00	0.00	0
Toxoid, 1% alum, 5% NaCl at room temp	9.8	0.78	12.5	0.32	0.41	31
Toxoid, 1% alum, 5% NaCl in incubator	9.8	0.80	12.2	0.30	0.37	33
Toxoid, 1.5% alum, 5% NaCl at room temp	10.1	0.69	14.6	0.24	0.35	42
Toxoid, 1.5% alum, 5% NaCl in incubator	10.1	0.81	12.4	0.23	0.28	44
Toxoid, 1.5% alum, 5% NaCl at 60° C	8.75	0.74	11.8	0.26	0.25	34
Diphtheria Toxoid, Experimental No 23 (Veal)						
Original toxoid (veal)	28 28.3	(4.40)	(63.64)	0.00	0.00	0
Toxoid, 2% alum at room temp	17.8 19	0.96	18.5 19.8	0.68	0.70	27
Toxoid, 2.5% alum at room temp	16.6 19	0.78	21.2 24.3	0.71	0.91	23
Toxoid, 2% alum in incubator	17.8	1.22	14.5	0.75	0.01	23
Toxoid, 2.5% alum in incubator	16.6	0.79	21.0	0.74	0.93	22

the material for immunizing purposes. The Al contents and the ratios given in the table are of interest. From the data given here, the most satisfactory conditions for processing the bulks of the toxoids shown in Tables I to III would be for the "veal" toxoid 2.5 per cent alum at room temperature, and for the "bacto-veal" toxoid 1.5 per cent alum, 5 per cent sodium chloride, and room temperature.

Employing the same conditions giving the best results shown in the final test in any given case, the alum-toxoid precipitate is prepared in large graduated bottles. In general, the following procedure is used. The toxoid is heated in a water-bath to 40° C, then taken out and the required amount, as shown by the tests, of sterile 10 per cent alum and in some cases sodium chloride is added. The bottle is shaken until the precipitate sediments completely. The supernatant fluid is siphoned off four hours later, replaced by sterile saline, and shaken. This is left to settle overnight. The following morning the washings are siphoned off and enough sterile saline is added to give the original volume of toxoid used. One to ten thousand merthiolate is added as a preservative. Throughout this procedure sterile technic is used in the addition of sterile saline and alum and in siphoning off the supernatant fluids to avoid contamination.

As a result of studying a considerable number of toxoid materials prepared here, the tests necessary for determining the most satisfactory conditions for the preparation in bulk of the toxoid-alum precipitate have been simplified. Whether such simplification applies to preparations elsewhere, cannot be stated at present. In general, the preliminary tests need be carried out only at room and at incubator temperatures for "veal" toxoids; with alum concentrations between 1.5 per cent and 2.5 per cent and no added sodium chloride; with alum concentrations between 1.0 per cent and 2.0 per cent and no added sodium chloride and with 5 per cent added sodium chloride for "bacto-veal" toxoids. The most satisfactory precipitates obtained in these tests can then be used for the final tests, and the best of these last used for bulk preparation.

A number of diphtheria toxoid alum precipitate preparations were analyzed. It would lead too far to present the detailed results. A general summary will therefore be given.

Nitrogen contents:

Original "Veal" and "Bacto-Veal" Toxoids	4.20 - 4.50 mg.N per c.c.
Toxoid alum precipitate, veal	0.90 - 1.41
Toxoid alum precipitate, bacto-veal	0.31 - 0.62

Aluminum contents:

Toxoid alum precipitate, veal	0.61 - 0.76 mg.Al per c.c.
Toxoid alum precipitate, bacto-veal	0.23 - 0.32

In every case, the volume of the toxoid alum precipitate was the same as that of the original toxoid material. The nitrogen and aluminum contents of the "veal" toxoid precipitate materials averaged two or more times as great as that of the "bacto-veal" toxoid precipitate materials. Whether these relations have any practical significance for immunizing purposes cannot be stated. The biologic potencies (Lf per c.c. or Lf per mg. N or Lf per mg. Al) naturally depend upon the original potency of the toxoid. In general terms the loss in the precipitation was 20 per cent to 40 per cent, independent of the original value.

Only in very few instances over a period of two years was it found necessary to add salt to large lots of diphtheria toxoid to precipitate with alum. Some of the toxoids made of bacto-veal were the only ones which showed such a need for salt, because the addition of alum alone failed to yield visible results. On the whole, the toxoids made with fresh veal gave a more uniform reaction to the addition of alum. After using the fresh veal toxoid for a time, to the exclusion of the bacto-veal, more uniform results have been obtained with regard to fineness of texture, lighter color, and much more uniformly dispersed precipitate.

#### CONCLUSIONS

As a result of the many tests on diphtheria toxoid precipitation, the following conclusions may be drawn:

1. Diphtheria toxoid made with fresh veal broth gave a better product as far as color, fineness, and uniform dispersion of the precipitate was concerned.

2 In general, 2 per cent alum concentration was needed for the maximum precipitation of fresh veal toxoids, while 1.5 per cent alum concentration gave the maximum precipitation with the bacto veal

3 If the toxoid did not precipitate by the addition of alum alone, the addition of solid sodium chloride brought about the precipitation

4 A better product was obtained when the toxoid was heated to 40° C prior to precipitation

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## THE MORPHOLOGIC SUGAR METABOLISM IN THE HUMAN LEUCOCYTE CULTURE\*

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THE morphologic representation of the sugar metabolism is only possible by the observation of the glycogen within the cells. The other products of carbohydrate metabolism do not give a specific histologic reaction. Thus, a complete representation of the whole carbohydrate metabolism cannot be brought about by histologic methods. Nevertheless the representation of the glycogen is sufficient for establishing differences of carbohydrate metabolism under different research conditions. The histologic glycogen reaction is a very sure one since Best has given us his new method.

The sugar metabolism in terms of changes in glycogen storage in the different cells has been presented by several authors. Pflüger, especially, has given us a distinct description of the connection between glycogen and administered carbohydrates. But all these researches have been made only on the liver cells of the living organism. The living organism is subject to very different influences which cannot be governed by us in our researches. We have tried, therefore, to examine the sugar metabolism according to the changes in the glycogen deposition in the human leucocyte. At first, we wished to study the glycogen deposition in the normal leucocyte culture and then after administration of different carbohydrates. Finally, we have examined the changes of the glycogen metabolism after addition of the different substances which govern the sugar metabolism, such as insulin. Under these circumstances, we hoped to obtain a complete picture of the carbohydrate metabolism in the human leucocyte culture. The observations have been made daily.

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The technical method of preparing the leucocyte cultures is described by us in a former work on the metabolism of the vital dyes in the human leucocyte culture. The demonstration of glycogen in the culture has been effected in that the whole culture was covered by a fine layer of collodion after fixation with alcohol, so that the glycogen itself could not be dissolved in water. Then we have proceeded in the manner described by Best.

At first, we observed the sugar metabolism of the normal human leucocyte culture as we find the carbohydrates in the normal human plasma. Without addition of any substances, certain glycogen deposits are already visible in the cultivated cells, just as they are in the normal leucocytes of the circulating blood. But in our researches, we have always convinced ourselves that the leucocytes did not show any glycogen granulations before the cultivation. As to the glycogen deposition in the normal leucocyte culture, we have found that, on the first two days of cultivation, a real glycogen storage is visible in the emigrating leucocytes. Both the macrophages and the granulocytes are now full of glycogen. But on the third day, the glycogen synthesis has been completed. In the next days, the cells show themselves free of glycogen. It may be concluded that, from that period, the blood sugar of the medium is consumed.

Sugar assimilation by the cultivated cells is also visible after addition of the different carbohydrates. Under these circumstances, the glycogen storage may be higher or lower in comparison with the glycogen metabolism in the normal leucocyte culture. The administration of carbohydrate may stop cell emigration and diminish glycogen storage.

The addition of glucose scarcely causes any glycogen deposition, there being only very scant amounts in the macrophages on the fifth day. Lactose does not effect a storage of glycogen at any time. But after addition of dextrose the glycogen deposition is more distinct. It makes its appearance especially in the macrophages but, in the last days, these cells also are free from glycogen. After fructose, we observe a complete absence of glycogen assimilation, also after addition of galactose and dextrin, while the glycogen synthesis is very insignificant after starch. Even the administration of alcohol does not effect a strong glycogen deposition in the human leucocyte culture. The glycogen is found in smaller quantities than in the normal culture without any additions to the medium. The glycogen deposition after maltose is only visible on the third day of cultivation.

On the other hand, we have found two kinds of sugar which effect very strong glycogen deposition in the cultivated cells. After administration of glycogen itself, we can establish a real and strong glycogen storage. Glycerin also causes a strong storage. We think that this is not a simple storage of the added glycogen itself. On the fourth day, we find glycogen as fine granules within the cells while larger granules are to be seen on the fifth day of cultivation. After administration of glycerin, glycogen is visible very strongly until the fifth day of cultivation.

Consequently, it develops from our researches that glycogen synthesis from the different sugars is brought about only under certain circumstances, that only

a few sugars are capable of building up glycogen. Many other sugars do not cause glycogen synthesis, or build up only very small quantities of glycogen. We cannot conclude that after these latter sugars, the sugar consumption is reduced, for there is the possibility that the sugar metabolism ensues in another way.

There are different hormones and irritating substances strongly influencing the sugar metabolism of the living organism, so that an augmentation or a diminution of the sugar metabolism takes place. We have examined insulin and thyroxin as to their influence on sugar metabolism in the human leucocyte culture, also adrenalin and tonephin. Helpine represents a leechin emulsion which has an insulin like action upon sugar metabolism (Magath). Finally, we have examined yeast which substance possesses a similar action and therefore is employed for the treatment of diabetes.

At first we examined the influence of these irritating substances without addition of any sugars to the medium. These substances did not cause any glycogen synthesis in the cultivated cells. The glycogen storage was less pronounced than under completely normal influences. Only under insulin and tonephin could we find a real glycogen storage on the second day. But on the following day, these storages had completely disappeared.

When to the human leucocyte cultures we added sugars together with these hormones, the reactions were of another character. We have already mentioned that the administration of glucose diminishes the glycogen storage in the leucocyte culture. Now, under the influence of insulin, very distinct storage of glycogen becomes evident. Helpine caused a more pronounced glycogen storage within these cells. Especially the macrophages show these metabolic products, but storage persists only until the third day. The other stimulating substances do not cause any glycogen storage in the cultivated leucocytes after addition of glucose itself.

After the isolated administration of lactose, no glycogen storage is evident. After addition of the different stimulating substances, no change in the microscopic sugar metabolism was observed. Only heated insulin and yeast extract cause the production of very fine glycogen granules for a few days. Dextrose has effected very distinct glycogen deposition after its addition to the medium. But under simultaneous action of insulin, glycogen storage becomes very strong. In the first day, glycogen deposition appears especially in the macrophages, where the nucleus may be completely covered by that substance. On the third day, deposition is evident only as fine granulation. Afterward, no more glycogen deposition is evident. A very remarkable fact in these researches is the augmentation of glycogen deposition in the cultivated cells after helpine. Our other stimulating substances have not caused a complete disappearance of glycogen storage. Fructose does not effect any storage. Under the influence of insulin, a very strong glycogen deposition is evident, especially on the third day. Sometimes a deposit in the macrophages is visible after addition of helpine, but not in such a degree as after insulin. After thyroxin, a very distinct glycogen deposition is evident in the macrophages only on the first day of cultiva-

tion. Maltose effected glycogen storage only on the third day. Under the action of most of our stimulating substances, these depositions could not be observed at all. Only under the influence of insulin and of adrenalin small depositions are evident in the macrophages.

Glycogen deposition under the influence of the glycogen itself is very strong as already mentioned. After addition of the other substances, we can observe a distinct diminution of glycogen deposition in nearly all cases. That fact is especially pronounced after addition of insulin and of helpine although these substances usually increase glycogen storages in the cells. Under the influence of glycogen, the strongest glycogen deposits were caused by simultaneous administration of thyroxin. Throughout nearly the whole time of cultivation, a very fine glycogen granulation was evident in the macrophages and in the granulocytes. After yeast and after adrenalin, no glycogen deposits were evident at all.

After addition of glycerin, deposition also was very strong, especially in the first five days. Under addition of insulin, the glycogen deposition is of the same character in the first days, the emigrating leucocytes showing a very heavy glycogen granulation. After helpine, storage is very unimportant and only visible for some short periods. The same results have been observed after thyroxin. Glycogen storage after yeast extract and after tonephin is only insignificant. Adrenalin does not effect any glycogen synthesis in these cultivated cells. After addition of alcohol, storage was very small in the cells. Only in the first days, glycogen granules were visible. After insulin, we did not find any glycogen storage in these cells during the whole time of cultivation. Adrenalin and tonephin did not effect glycogen storage in these cells. But very distinct glycogen granules were evident after helpine. Storages were more extensive than after insulin. The macrophages were as full of glycogen granules as the granulocytes in the first days. But afterward a complete disappearance of the glycogen deposition took place.

After simultaneous administration of insulin and galactose, glycogen storage increased strongly in all the periods. But from the sixth day, a complete disappearance was evident. Synthesis after helpine is very insignificant; it is a little stronger after thyroxin where especially the polynuclear leucocytes show very distinct glycogen depositions on the first and on the fifth days. Storage is insignificant after tonephin, adrenalin, and yeast extract.

Dextrine administration was not accompanied by glycogen deposition in the human leucocyte culture. Under the simultaneous influence of our hormones, glycogen metabolism did not change. Only under the influence of insulin could we observe some glycogen granulations in the granulocytes on the first day, while the other cells of the same culture were free; but in the following days we could not find any glycogen.

After administration of starch we could normally observe distinct glycogen granules within the macrophages on the third day. Under the simultaneous influence of our hormones we could not see such a reaction. Glycogen deposition was of a very strong character after insulin, especially in the macrophages. Sometimes storage was of so great an intensity as to cover all the other cell structures. After helpine complete absence of glycogen was evident. The same

microscopic findings were present for yeast tenephin, and adrenalin, but thyroxin effected a real glycogen storage in the macrophages and in the granulocytes during the whole cultivation time

So we can recognize in these researches that there is no uniform action of the stimulating substances upon the metabolism of the different sugars. After insulin, we observed an augmentation of the glycogen storage only after addition of sugars such as glucose, dextrose, fructose, galactose, while, after addition of other sugars, an augmented glycogen synthesis is never observed. As to the action of insulin, this is not necessarily contradictory to its effect in the living organism, because we have applied only small doses of insulin under these circumstances. On the other hand morphologic insulin metabolism has been observed only in the liver cells of the whole organism. It is said that helpine shows an insulin like action. In our researches on the human leucocyte culture we established glycogen synthesis with helpine, sometimes more pronounced than that caused by insulin. This is especially noticeable after administration of glucose and of alcohol. As to the other sugars glycogen synthesis may be suppressed completely by helpine. Yeast is said to also show an insulin like action, diminishing the blood sugar in the living organism. In our researches, we observed that glycogen storage in the human leucocyte culture did not increase after yeast. Only in our series with lactose a transitory glycogen storage took place within the granulocytes.

There is a diminution of glycogen deposition in the liver cells after administration of thyroxin to the living organism. In the cultures there was an increase in glycogen after simultaneous administration of glucose although this synthesis appeared in the last periods of cultivation. An unimportant storage took place after thyroxin and the sugars glucose, fructose, galactose, and alcohol. Sometimes glycogen synthesis under thyroxin surpassed the storage after insulin. Glycogen storage is especially strong after addition of glycogen itself to thyroxin. The same results were to be found after starch.

We must answer the question if the absence of glycogen in the cultivated cells is to be regarded as a sign of an absent carbohydrate synthesis. We may think that a lack of glycogen reveals a very strong digestion of the carbohydrates, but other metabolic products may develop which are not visible by microscopic methods. Certainly, a negative finding as to the glycogen deposition can only be regarded as a negative finding for itself.

Nevertheless we feel that we have pointed out a new way for the study of carbohydrate metabolism. From these researches it results that the microscopic method reveals a great many new facts regarding the metabolism of the cells. Therefore, the microscopic method should be employed much more in the study of the metabolism of the organism.

#### CONCLUSIONS

The sugar metabolism of the human leucocyte culture may be studied by the morphologic observation of glycogen storage in the cultivated cells.

We have demonstrated that glycogen synthesis from the different sugars varies greatly. We observed strongest glycogen storage after administration of

glycerin and of glycogen itself. Insulin can be regarded as a glycogenetic substance in the human leucocyte culture only after simultaneous addition of certain sugars. Otherwise a diminution or no change of the glycogen deposit took place. Another substance of a certain glycogenetic activity was a lecithin emulsion, called helpine.

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### NEUFELD REACTION IN CERTAIN CASES OF PNEUMOCOCCIC SEPTICEMIA\*

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THE blood culture in some cases of pneumonia appears to offer the only medium of rapid determination of type. Random cases of infection which have entered this hospital for observation during the past two years have proved to be respiratory pneumococcal infection of atypical clinical course which could not be typed in the usual manner by means of sputum because in some expectoration was absent. Blood cultures, however, were positive for organisms of the streptococcal-pneumococcal groups within eighteen hours.

Diffuse growth of the organisms occurred in initial broth culture of the blood, yet attempts to determine type by means of bile solubility, macroscopic agglutination, or precipitin tests failed. Exhibition of failure of the tests was evidenced when the tubes were placed in water-bath and opaque yellowing precipitates settled out in a few minutes. These precipitates of nonspecific unknown identity interfered, and this interference arose apparently from the patient's blood stream constituents. They suggested heavy colloidal suspensions being thrown out of the dispersed state. The difficulty was eliminated by preparation of initial saline suspensions from centrifugalized sediment of the initial supernatant blood broth culture. Bile solubility and Neufeld tests were made from the saline suspensions. Neufeld tests were satisfactory in each instance, 100 per cent of the pneumococci showing encapsulation in specific serums. Mouse inoculation tests of such cultures were unnecessary, as well as preparation of subcultures for purpose of elimination of the interfering substances.

The precipitin test of Krumwiede and Valentine was performed upon sputums and blood culture cell sediment from many of the cases. For some

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unexplained reason, there have been few positive preeipitin tests for the past two winter seasons. This finding has been concurrent with atypical clinical pneumonias.

Preparation of saline suspension of all streptococcal-pneumococcal organisms isolated from blood were made for determination of type of pneumococci by means of bile solubility and Neufeld tests. The preeipitin test of Krumwiede and Valentine was set up as a comparative procedure. Immune rabbit serum for the tests were obtained from Miss G. Cooper of the Research Laboratories of the Department of Health, New York City, and from Lederle Laboratories of New York.

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## THE DOCTOR AS CONTRIBUTOR TO CIVILIZATION\*

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THE doctor's contribution to the welfare of humanity in extramedical spheres are many and of the greatest value and interest. As explorer, as philosopher, as sociologist, as musician he has contributed to the advance of progress. In this article we shall review some of these many-sided activities of the doctor.

### THE DOCTOR AS EXPLORER

The earliest of great doctor-explorers was Mungo Park, born in 1771. At fifteen years of age he was apprenticed to a surgeon, Thomas Anderson by name, under whom he studied for several years. He later continued his medical studies at Edinburgh where he obtained his medical degree. In 1791 he went to London where he obtained an appointment as assistant medical officer on the "Worcester." In 1792 he sailed to the East Indies, returning one year later with rare plants and animals from Sumatra which he presented to his patron, Sir Joseph Banks.

By 1795 the reputation of Mungo Park as an explorer was fully established. In that year he was sent by the African Association to find the course and termination of the Niger River. He had many adventures on that voyage of exploration. In July of that year he arrived at Pisiana, 200 miles up the Gambia. Six months later he started on a journey of adventure and discovery, accompanied by a negro servant and a boy. He was robbed by the natives through whose territory he passed. He was lucky to get away alive. For four months he was kept a prisoner by an Arab chief, from whom he ultimately escaped.

Mungo Park was the first extensive explorer in the mysterious continent of Africa. He was the first European of modern times to strike the Niger and his work, more than any other's, did most to open up the dark continent to exploration and settlement by the white man.

The most famous of doctor explorers was David Livingstone (1813-1873) who began life in a cotton factory at the age of ten. His interest in medical

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missionary work asserted itself early. He enlisted with the London Missionary Society and studied medicine in London, becoming a licentiate of the Faculty of Physicians and Surgeons of Glasgow in 1840.

Immediately upon graduation Livingstone sailed on the first of his three great trips of exploration to Africa. It was his great ambition to open up Africa to commerce and to abolish its slave trade evil. He wanted not only to show the advantages of Africa to the white man but also to better the conditions of the native black man.

Dr. Livingstone died at Ilala on Lake Bangweulu in 1873. His heart was removed from his body by his faithful native followers and buried under a tree. His embalmed and dried body was then brought after a long and hard journey to the coast whence it was sent to England to rest forever in Westminster Abbey.

Another notable doctor explorer was Sir John Richardson (1787-1865) who at the early age of twelve was apprenticed to his uncle who was a surgeon. Later he studied and qualified as a member of the Royal College of Surgeons in London. In medical practice he was not very successful and for that reason he applied for appointment as surgeon and naturalist to the first Arctic expedition of Sir John Franklin. He was second in command. From 1825 to 1827 Dr. Richardson was with Franklin on his second voyage of exploration. He was the first scientist to make a thorough study of Arctic flora and fauna.

#### THE DOCTOR AS PHILOSOPHER

To philosophy and psychology the doctor has been a significant contributor. John Locke (1632-1704) was one of the greatest of early English philosophers. He received his M.A. from Oxford in 1658, and he became a lecturer at that university in Greek and rhetoric. Later he decided to study medicine. In 1668 he became an F.R.S. and in 1674 he received his M.B. and practiced medicine for several years. He was an intimate friend of the great English clinician Thomas Sydenham and often accompanied him in his rounds. He wrote a Latin poem for the second edition of Sydenham's work on fevers.

Dr. Locke's greatest work is entitled *An Essay Concerning Humane Understanding*, which was published in 1690. Twenty editions of this remarkable work appeared by the end of the eighteenth century. It was translated into French, Latin, and German. Locke was recognized as the leading philosopher of his time. Another important book, *Epistola de Tolerantia*, was published in Latin. It was soon translated into other languages. Dr. Locke was one of the really great philosophers of all time.

In Germany Rudolf Hermann Lotze (1817-1881) held a similar position. He was a graduate of the medical faculty of Leipzig, but his interests lay mainly in philosophical studies in which he established an enviable reputation for himself.

In this country the medical profession is represented by William James (1842-1910) who was one of the greatest psychologists of modern times. William James studied medicine at Harvard from which he received his M.D., but he never practiced his profession. He at first taught anatomy and physiology at his Alma Mater, but later his interests were attracted to philosophy and psychol-

ogy, which subjects he also taught at Harvard. He became the Professor of Psychology in 1885. He is the author of an imposing list of very important books: *Principles of Psychology*, *The Varieties of Religious Experience*, *Pragmatism*.

#### THE DOCTOR AS MUSICIAN

Among the earliest of noted English composers was George Ethridge who lived during the sixteenth century and who was one of the most famous vocal and instrumental musicians of his day. He was a graduate of Oxford and a physician of no little ability.

Among the earliest compositions extant by medical men are three songs by Thomas Campion. These are dated 1596 and are in the Harleian Manuscripts 6910 (British Museum). Campion took the degree of Doctor of Medicine, apparently at Cambridge and had previously studied for the legal profession. He also published four books of *Aires* which have given him a place in musical biography. He was also a musical theorist of note, his *New Way of Making Four Parts in Counterpoint, by a Most Familiar and Infallible Rule*, published about 1618, went through many editions.

Twenty years before Campion's death the will of Sir Thomas Gresham, establishing a Professorship of Music had taken effect. The first five men to hold this chair were all physicians.

Probably the medical man whose name is most familiar to music lovers is Thomas Harington. His *Great Is the Pleasure* is one of the most popular of musical compositions, and it has been played and sung in all quarters of the globe. He was born in 1727 at Kelston Somerset, England. He had at first intended to study for the Holy Orders but later changed his mind and qualified as M.D. at Oxford. During his college years he became very much interested in music.

Dr Harington was most successful in all his undertakings. His life was spent chiefly in Bath where he was a prominent physician. He also became much interested in civic affairs and was first alderman and later mayor of his city. All his leisure was devoted to music and composition. He composed much music during his lifetime, most of which became very popular.

Another early English doctor composer was William Kitchener who was born in 1775 and who took his M.D. at Glasgow University. Dr Kitchener composed not only songs but also more ambitious works. He composed a musical drama under the name *Ivanhoe* and an operetta *Love Among the Roses*. He died in 1827.

By no means were the doctor musicians limited to England. Florent Corneille Kist, born at Arnheim, Holland, in 1796, studied medicine and practiced as a doctor at The Hague until 1825, after which he devoted himself entirely to music. His compositions consist of vocal pieces for one and several voices, and a volume of variations for the flute on which he was an excellent performer. But it is chiefly as an organizer of musical societies at Delft, The Hague, and Utrecht and founder of the *Caecilia*, which is still the most important musical paper in Holland, that he is known.



## OTHER ACTIVITIES

The extramedical activities of physicians have been most varied and significant. There is scarcely a field of human endeavor which has not felt the touch of the doctor's hand. Fire insurance was originated by a medical man. Nicholas Barbon was born in London and studied medicine at Leyden. In 1661 he graduated (M.D.) at Utrecht and three years later he was admitted to the fellowship of the College of Physicians. For a time he was a member of Parliament. After the Great Fire of 1666 he was one of the principal builders of the city of London. What he is most remembered for was that he was the first projector of fire insurance.

One of the great pioneers of South Africa was William Guybon Atherstone (1814-1898). It was from his researches that the great diamond mining activity in South Africa was initiated. In 1867 he identified the first diamond found at Colesburg-Kop, now known as Kimberley. Dr. Atherstone examined the diamond with a polariscope and tested its hardness on a windowpane. The diamond was later sold for \$4,000. The original windowpane upon which the diamond was tested has been framed and is still preserved as a memento of that great occasion. In 1888 the Kimberley companies presented the pioneering physician with a valuable diamond as a token of appreciation for his work in founding a great industry.

To chemistry, doctors have made some very important contributions. As space is limited only three will be referred to. Thomas Andrews was the first to demonstrate the true nature of ozone, proving it to be an allotropic form of oxygen. Joseph Black is immortalized as the discoverer of latent heat in 1761. He also originated the theory of specific heat. In 1767 Dr. Black made the first attempt to inflate a balloon with hydrogen.

Another notable chemist was Daniel Rutherford (1749-1819) who was born in Edinburgh and received his medical education from the university in that city. His thesis for the M.D. degree in 1772 was significant because of the clear distinction he made between carbonic acid gas and nitrogen. He was also the first to isolate the gas nitrogen by burning substances in an enclosed volume of air and absorbing the carbonic acid thus formed.

By no means are the extramedical activities of doctors covered in this and the preceding articles. Nor have all the names been mentioned. To do this would have been a most ambitious undertaking and a most comprehensive work. These articles have served merely to call attention to the fact that doctors have made great contributions not only to medicine but to almost every field of human endeavor. The extramedical contributions of the medical profession have been almost as important, perhaps just as important, as their purely medical contributions.

## HEMATOLOGIC NOMENCLATURE\*

"TEKNOCYTE" AND "KOROCITE" SUGGESTED IN EMENDATION OF THE DESIGNATIONS "JUVENILE" AND "STAB" IN THE SCHILLING HEMOGRAM

ARTHUR PARAEER HITCHENS M D WASHINGTON D C

THE validity and practical worth of Schilling's<sup>1</sup> contribution to the clinical study of infections and intoxications are being constantly more widely recognized. Simplicity is often mentioned as one of the points in its favor just as complexity may have caused neglect of its predecessor the Arneth<sup>2</sup> count. Much more important however are differences of interpretation based upon the regenerative and degenerative influences revealed by the appearance and number of the ontogenetic types of neutrophils enumerated. Arneth grouped his three young cells (M W T) and separated the various mature neutrophils in accordance with their nuclear structure. Schilling on the other hand separated Arneth's three young types with minor differences in cleavage and grouped the polymorphonuclear neutrophils thus he had the basis for an analysis of the influences which alter not only the development but also the production of the granulocytes.

The several attempts that have been made to reduce the time consumed in making complete Schilling hemograms have not been quite successful, because valuable information has been sacrificed to save a negligible amount of time. All cells present must be counted and any abnormality of appearance noted, whether the tally is put down in one column more or less can make little difference. In any laboratory the blood specimens in which there is a marked "shift to the left" will usually be exceptional, when such do occur the information gained by careful study is worth more to the clinician than it costs in extra time. This point (of the relative negligibility of the time factor) receives emphasis from the work of those who are restudying intensively the cytoplasmic changes in the granulocytes.<sup>3,4</sup> That any consolidation of the three young forms of Arneth (M W T) or of Schilling (myelocyte, juvenile, stab) may reduce the value of the count disproportionately is made clear by Piney.<sup>5</sup> If further evidence is needed to support this statement it is furnished amply by study of the figures in the first three columns under Class I, of Arneth's original protocols (although only the granulocytes are counted) with their respective case histories. Schilling's own work is still more convincing, of course. Appreciative notation might also be made here of the recent paper by Crocker and Valentine.<sup>10</sup>

However, there is a point in connection with the Schilling hemogram which demands emendation. This is the matter of nomenclature. The language of hematology has otherwise remained remarkably free from the taint

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of medical slang. In the past, writers have not hesitated to apply accurately descriptive terms, of Greek derivation, to the blood cells as shown by the current use of such words as "poikilocytosis" and "anisocytosis."

Even though the words "juvenile" (jugendlich) and "stab" have been in use since 1912,<sup>1</sup> when the first edition of Schilling's work was published in Germany, and especially since 1929 when Gradwohl's<sup>2</sup> translation of the seventh and eighth revised edition was published in this country, they cannot be considered as anything but a part of the convenient and careless argot of the laboratory. Furthermore, they have by no means found general acceptance.

Whether or not there is universal agreement as to the technique, significance and limitations of the Schilling differential count, or whatever modification of it the future may bring about, the morphologic types called by Schilling "juvenile" and "stab" represent significant developmental stages in maturation between the myelocyte and the polymorphonuclear leucocyte. As such they deserve designations in conformity with the nomenclature of biology, designations, which, strange to say, they have never received.

In seeking suitable names we have no fixed precedent as to the important characteristic of a blood corpuscle which shall be represented. The words myeloblast and myelocyte refer to the place of origin of these cells, with "blast" and "cyte" designating their respective ages or state of maturation. "Polymorphonuclear" describes the most striking and characteristic appearance of this mature leucocyte, while "neutrophilic" (heterophilic),<sup>11</sup> "eosinophilic," and "basophilic" refer to chemical reactions of cytoplasmic granules in the respective formed elements of the blood.

So far as their significance in clinical pathology is concerned the important characteristic of the "juvenile" and "stab" cells is their developmental age. The younger of the two, the "juvenile" of Schilling, in its age relationships is like the Greek word τέκνον: that which is born, a child; the somewhat older cell, but still immature in the form and shape of its nucleus, the "stab nuclear" of Schilling, seems to correspond in relative age with the Greek κόρος: a boy, a lad, up to the military age.

The adoption of the following into the nomenclature of hematology is therefore recommended:

Teknoocyte (τέκνον + κύτος), the young granulocyte, rarely found in the circulating blood in health (0-1 per cent), which follows ontogenetically immediately after the myelocyte. It is somewhat larger than the mature cell; its protoplasm is like that of the older granulocyte; the nucleus varies in form from sausage shape nearly to bean shape. The chromatin does not stain quite so deeply as that of the mature cell but it is distinctly subdivided into fields, with definite nuclear bodies in the end bulbs (circumscribed chromatic nucleoli). It is identical with the Schilling "juvenile neutrophilic leucocyte."

Koroocyte (κόρος + κύτος), the youngest granulocyte found regularly in the circulating blood in health (3-5 per cent). In ontogenetic development it is between the teknoocyte and the polymorphonuclear. In size it is barely larger than the mature granulocyte and the cytoplasm of both is alike in

appearance. The nucleus is narrower, more shriveled, than that of the teknocyte and in shape may resemble the letters and figures C, U, V, S, Z, W, T, E, 6, 8, 3. Segmentation is never so nearly complete as in the polymorphonuclears, the lobes are connected by bands broader than a thread. The margins are uneven and the chromatin is gathered in irregular, dark compact masses between which light apertures appear. There are no nucleoli. It is identical with the Schilling "stab nuclear leucocyte."

The degenerative korocyte (degenerative Stabkeimige) is a neutrophile which due to degenerative inhibition of development has matured without segmentation. It is distinguished from the normal korocyte by cytoplasmic differences and by its narrow hyperchromatic and pyknotic nucleus which is sometimes curved into bizarre shapes. This cell is considered of special significance in the hemogram. Recognition of degenerative changes in it does not, however, preclude observation of such changes in the other cells as have been so carefully recorded by Naegeli<sup>19</sup> and Gloor.<sup>20</sup>

In common with the other granulocytes teknocytes and korocytes may be neutrophilic, eosinophilic or basophilic.

These young cells were among those described and discussed by Ehrlich.<sup>21</sup> They were known as transitional forms of the "c" granulocytes but no one had attempted an orderly arrangement of them prior to the work of Arneth.<sup>2</sup> His great contribution to hematology was the result of careful morphologic studies which culminated in the "Arneth count." This as is well known correlates the differential count of the various nuclear forms in the circulating blood, with disease. The proportions of old and young forms were so related to infectious conditions that the count was of value not only in diagnosis but in prognosis as well. He used the term "shift to the left" to denote the increase in young forms which he found to occur regularly in severe pyogenic infections and certain other conditions. He separated the neutrophils into five classes according to the configuration of their nuclei. The three youngest forms he grouped together as Class I, designating the myelocyte as "M," the younger of the other two cells as "W" (Zellen mit wenig eingebuchtetem Kerne), the older as "T" (Zellen mit tief eingebuchtetem Kerne). Pappenheim<sup>22</sup> had concerned himself more with the ontogenesis of the blood cells than with their relationship to clinical pathology. To him the group of forms included under Arneth's "W" and "T" types were merely cells which follow the myelocytes in development toward maturity. Therefore, all the intermediate forms he called "Arneth cells" or "metamyelocytes," (*μετα*—after). He also called the cells immediately preceding the myelocytes in age "promyelocytes," (*πρo*—before).

Pappenheim's interest was purely scientific, Schilling's, like Arneth's, was practical, the application of blood counts to disease. Therefore Schilling agreed with Arneth in recognizing two intermediate types or two age forms instead of one. Schilling at first<sup>23</sup> considered the "juvenile" forms identical with metamyelocytes but later<sup>24</sup> stated that "They are nearly identi-

<sup>23</sup>The description of the teknocyte follows closely that given by Schilling<sup>2</sup> while details of the korocyte are taken largely from Crocker and Valentine.<sup>25</sup>

cal with Pappenheim's metamyelocytes, which, however, are not clearly outlined by the author." Study of various forms in Pappenheim's Atlas,<sup>13</sup> inclines one to agree with this statement, since the cells shown as metamyelocytes cover the entire range between myelocyte and polymorphonuclear. With regard to his "stab nuclear" Schilling does not agree that it is identical with Arneth's T; he seems to consider the younger of W's to be true myelocytes and the older to be stabs. The lack of agreement between Arneth and Schilling upon these points is probably no greater than between any other two hematologists concerning the Schilling types. There can be no sharp, natural, differentiating criteria because the forms in question are developmental stages. There is need for cooperative work by a committee of interested persons to establish the characteristics of these two important types and eliminate as far as possible local differences among the accepted pattern cells of the various laboratories.

Uncertainty as to differentiation has undoubtedly had much to do with suggestions that the teknocytes and koroocytes be counted together as metamyelocytes, or by some other name. Furthermore, nearly every worker, unable to see a band or a rod in the stab (!) shaped nucleus, has invented his own designations. None of the names suggested has been constructed in accordance with the traditions of biologic nomenclature; a few of them are tabulated below. The upper part of the table is an adaptation of one arranged by Schilling.<sup>14</sup>

TABLE I

	MYELOCYTES	TEKNOCYTES	KORO CYTES	POLYMORPHO- NUCLEARS
			DEGENERATIVE KORO CYTES	
Schilling <sup>1, 14</sup>	Myelozyten	Jugendliche	Stabkernige	(Segment kernige
			Degenerative Stabkernige	
Gradwohl <sup>2</sup>	Myelocytes	Juveniles	Rod or Staff nuclears	Segment nuclears
			Degenerative stab or staff forms	
Arneth; <sup>3</sup> Schilling <sup>14</sup>	M	W	T	Segmented Classes II-V
Pappenheim <sup>13</sup>	Myelozyten	Metamyelozyten		Polymorpho- nuclearen
Cooke and Ponder <sup>15</sup>	-	-	Class I	Class II-V
Piney <sup>9</sup>	Myelocytes	Young forms of metamyelo- cytes	Band forms of metamyelocytes	Polymorphs
Pons and Krumbhaar <sup>16</sup>	Metamyelocytes (very young)		Nonsegmented forms (young)	Segmented forms (older)
Farley, St. Clair, and Reisinger <sup>17</sup>	Myelocytes	Nonfilament nuclear types		Filament nuclear types
Kolmer and Boerner <sup>18</sup>	Myelocytes ?	Y or young types	B or band types	Neutrophiles
Crocker and Valentine <sup>10</sup>	Myelocytes	Juveniles	Stabs	Segmenters

In the volume by Cooke and Ponder,<sup>15</sup> as Fig. 2, is "a graphic representation of Arneth's scheme taken from Gruner's *Biology of the Blood Cells*." This drawing is interesting because it shows that, as interpreted by some

workers, there was no correlation between the Arneth types and those of Schilling. Cells considered as Class III of Arneth would be called stabs by Schilling. Cooke's modification of the Arneth count is also illustrated in this volume. No cell younger than a koroocyte is shown.

One of the methods suggested to simplify the Arneth count is that of Pons and Kumbhaai.<sup>16</sup> They divide the neutrophils into "(1) metamyelocytes (very young), with round or slightly indented nuclei, (2) nonsegmented forms (young), where the nuclear material is connected by broad bands, (3) segmented forms (older), where two or more groups of nuclear material are joined by narrow filaments." The illustration shows, as Group 1, forms which would generally be called myelocytes, in Group 2, the forms pictured are similar to those shown by Pappenheim<sup>12</sup> and called by him "metamyelocytes", some of them would be called by Schilling "juvenile" and the others "stabs". The reason for suggesting such a "shift" in terminology is not clear.

More recently, Farley, St. Clair, and Reisinger<sup>17</sup> have suggested a simplification of previous methods by dividing the polymorphonuclear neutrophils into "filament" and "nonfilament" nuclear types. This is said to be based more directly upon the work of Cooke and Ponder.<sup>10</sup> They reprint the illustration from Cooke and Ponder which shows Class 1 to be composed of koroocytes. The text states however, "Distinct myelocytes or myeloblasts are to be counted as such."

In the volume, *Approved Laboratory Technique*, prepared under the auspices of the American Society of Clinical Pathologists, Kolmer and Boerner<sup>18</sup> state, on p. 82, "Schilling has recommended a division of the metamyelocytes into two types, namely, (a) young forms with round or slightly indented nucleus corresponding to Arneth's M and W cells and (b) band forms with deeply indented or bandlike nuclei which correspond to Arneth's T cells (Fig. 60)." Actually, Arneth<sup>3</sup> used the 'M' as the initial letter of the word "myelocyte"—"M soll Myelocyt zu bedeuten" (p. 17). These authors (p. 82) suggest "Y" and "B" as designations for the young granulocytes. "Namely, (a) those with a slightly indented nucleus called young or Y types and (b) those with a deeply indented nucleus called band or B type." It is not quite certain, for Fig. 66 offers no help but it seems that this classification resembles Schilling's and not that of Pons and Kumbhaai<sup>16</sup> as the statement concerning Schilling's division of the metamyelocytes prepares one to expect.

This brief review is believed sufficient to indicate the carelessness that has crept into hematologic nomenclature and literature and to emphasize adequately the need for a return to classic terminology and an orderly scientific language.

#### SUMMARY

1 The methods suggested as substitutes for the Schilling hemogram save an insignificant amount of time at the expense of valuable information.

2 The immature granulocytes called by Schilling "juvenile" and "stab nuclear" have never received designations in conformity with scientific hematologic nomenclature.

cal with Pappenheim's metamyelocytes, which, however, are not clearly outlined by the author." Study of various forms in Pappenheim's Atlas,<sup>13</sup> inclines one to agree with this statement, since the cells shown as metamyelocytes cover the entire range between myelocyte and polymorphonuclear. With regard to his "stab nuclear" Schilling does not agree that it is identical with Arneth's T; he seems to consider the younger of W's to be true myelocytes and the older to be stabs. The lack of agreement between Arneth and Schilling upon these points is probably no greater than between any other two hematologists concerning the Schilling types. There can be no sharp, natural, differentiating criteria because the forms in question are developmental stages. There is need for cooperative work by a committee of interested persons to establish the characteristics of these two important types and eliminate as far as possible local differences among the accepted pattern cells of the various laboratories.

Uncertainty as to differentiation has undoubtedly had much to do with suggestions that the teknocytes and korocytes be counted together as metamyelocytes, or by some other name. Furthermore, nearly every worker, unable to see a band or a rod in the stab (!) shaped nucleus, has invented his own designations. None of the names suggested has been constructed in accordance with the traditions of biologic nomenclature; a few of them are tabulated below. The upper part of the table is an adaptation of one arranged by Schilling.<sup>14</sup>

TABLE I

	MYELOCYTES	TEKNOCYTES	KOROCYTES	POLYMORPHO- NUCLEARS
			DEGENERATIVE KOROCYTES	
Schilling <sup>1, 14</sup>	Myelozyten	Jugendliche	Stabkernige	Segment kernige
Gradwohl <sup>2</sup>	Myelocytes	Juveniles	Degenerative Stabkernige	
			Rod or Staff nuclears	Segment nuclears
			Degenerative stab or staff forms	
Arneth, <sup>3</sup> Schilling <sup>14</sup>	M	W	T	Segmented Classes II-V
Pappenheim <sup>13</sup>	Myelozyten	Metamyelozyten		Polymorpho- nuclearen
Cooke and Ponder <sup>15</sup>	-	-	Class I	Class II-V
Piney <sup>9</sup>	Myelocytes	Young forms of metamyelo- cytes	Band forms of metamyelocytes	Polymorphs
Pons and Krumbhaar <sup>16</sup>	Metamyelocytes (very young)		Nonsegmented forms (young)	Segmented forms (older)
Farley, St. Clair, and Reisinger <sup>17</sup>	Myelocytes	Nonfilament nuclear types		Filament nuclear types
Kolmer and Boerner <sup>18</sup>	Myelocytes ?	Y or young types	B or band types	Neutrophiles
Crocker and Valentine <sup>10</sup>	Myelocytes	Juveniles	Stabs	Segmenters

In the volume by Cooke and Ponder,<sup>15</sup> as Fig. 2, is "a graphic representation of Arneth's scheme taken from Gruner's *Biology of the Blood Cells*." This drawing is interesting because it shows that, as interpreted by some

# LABORATORY METHODS

## A NEW METHOD FOR THE DETERMINATION OF CUTANEOUS CAPILLARY BLOOD PRESSURE\*

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MANY methods are available for the determination of the blood pressure in capillaries. The most direct method is probably that of Carrier and Rehberg<sup>1</sup> in which a minute tube filled with saline connected to a manometer is inserted directly into a capillary and the entrance of blood or exit of salt solution at different pressures is noted. This method while direct is only suitable for research purposes. The method of Danzel and Hooker<sup>2</sup> is also excellent but requires rather elaborate equipment (Duryee and Wright<sup>3</sup>). These authors observe through a transparent pressure capsule the movements of corpuscles in individual capillaries. The pressure found necessary to stop

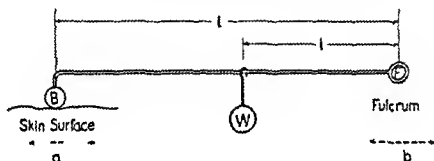


Fig. 1

the flow of corpuscles in the capillaries is found to be very much greater than the pressure that produces paling of the skin. Paling of the skin is used in the method of von Kries<sup>4</sup> which consists essentially of weighting a glass of known area until the capillaries' bed under the glass is bleached. This method is subject to the criticism that during the loading period the capillary bed is more or less occluded and subject to stasis of the blood and in addition, the area of glass applied to the skin is variable due to the topography of the skin. Other methods are based essentially on this same method and are subject to error due to the resistance of the cutaneous tissues to deformation.

The new method about to be described has yielded results comparable to those of Carrier and Rehberg. Such results have been obtained by students as well as by trained investigators. The instrument (see Fig. 1) consists of an arm hinged at one end to an upright and bearing on the other end a small glass bead. The arm is bent downward at right angles to carry the bead. In operation the glass bead (B) is applied to the skin by a sliding weight (W) on the calibrated arm (l). The skin surface is moved, as indicated by arrows a,

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at a moderate speed with the glass ball riding over the skin. When the skin surface itself cannot be easily moved, the ball may be made to make excursions back and forth (*b*) by having the fulcrum (*f*) of the lever on a ratchet and pinion. If a white flash appears, as the ball passes by, the weight is moved nearer the fulcrum until the pink color of the skin is undisturbed by the weights. These white flashes are of very short duration and are not to be confused with the local vasomotor reaction to strong mechanical stimuli, which has a latent period and duration of many seconds (Krogh<sup>5</sup>).

The actual surface of the ball that makes contact is determined by noting the diameter of the ink spot on the ball after application to the ink-smearred skin. This area divided into the pressure, calculated by the law of levers, gives a value convertible into mm. Hg.

$$\frac{Wl'}{l} + \frac{\text{wt. lever}}{2} = P$$

$$\frac{P \text{ in milligrams}}{\text{area in sq. mm.} \times 0.0136} = \text{mm. Hg}$$

The method avoids the danger of stasis, gives a statistical average surface that is constant as the ball passes rapidly over cutaneous areas of different contour and variable deformability. The adjustment of pressure is convenient and accurate.

The importance of determination of capillary blood pressure in clinical work may be expected to increase. Already this measurement is of importance in urticaria, inflammation, circulatory shock, edema, etc. (Krogh<sup>5</sup>).

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## THE LIMITATIONS OF COLORIMETRIC ANALYSES BY PRESENT METHODS\*

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IN DOING routine colorimetric determinations, the authors have been impressed by the frequent discrepancies in readings obtained with slight differences in technique. The following data are an attempt to create a more general appreciation of the inherent limitations to the accuracy of various routine colorimetric analyses. These limitations are the result of many chemical factors, the most important of which are correlated in Table I. The data presented in Table I have been selected from many hundreds of analyses made over a period of a year. The test results selected from this large group were chosen because they best illustrate the factors of error involved and each represents an average reading resulting with the correlated factor. The figures and notes have been arranged, purposely not in chronologic order, but rather in a sequence which tends to show the cumulative effect of these prominent factors.

We might digress to explain that colorimetric chemistry is that science which deals with the quantitative analysis of unknown chemicals in terms of color reactions. These color reactions are derived by a special treatment of the substance under analysis with a reagent which is often very complicated and which produces a color reaction whose intensity varies in relation to the quantity of the substance being analyzed. This method of quantitative chemical analysis, although in general a relatively simple procedure for the clinical technician, nevertheless involves many technical factors (often beyond the control of the operator) which influence the degree of precision of the readings. These types of analyses even if handled with the extreme care and caution of a skilled analytical chemist are not free from some erratic results. The following exemplary data taken from an experimental notebook will tend to show some of the factors normally overlooked by the routine clinical technician.

The data were obtained through the use of a new type of photo electric colorimeter of the author's design, the advantages of which will be described below. With this instrument it was possible to obtain color density readings without intercomparison with a standard, thus making it possible to measure accurately the influence of such factors as treating technique, temperature, time, concentration of reagents etc. It can be indisputably proved (see description of colorimeter) as far as these data are concerned that the precision of the color density readings of this instrument was of a much higher order of accuracy than the errors or percentage differences which are shown in the table. These errors, therefore, are essentially the result of some erratic chemistry, which the authors

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have attempted to correlate with the major causative factors. It should be obvious from this table that the reaction technic alone which involves the factors under columns five and six may routinely introduce errors of at least 5 to 10 per cent.

TABLE I

ACTUAL WT. OF SPECIMEN	NO.	EQUIVALENT COLOR DENSITY READING	% OF ERROR	SIZE OF CONSTRICTED NECK OF FOLIN TUBE	AGITATION AFTER REDUCTION OF CU
BLOOD SUGAR					
200.0 mg.	A	190.0	- 5.0%	Average	Normal routine care
200.0 mg.	B	190.0	- 5.0%	Average	Normal routine care
150.0 mg.	C	140.0	- 6.6%	Average	Normal routine care
125.0 mg.	D	122.0	- 2.4%	Average	Normal routine care
100.0 mg.	E	91.0	- 9.0%	Slightly larger	Normal routine care
100.0 mg.	F	91.0	- 9.0%	Slightly larger	Normal routine care
100.0 mg.	G	89.5	-10.5%	Slightly larger	Normal routine care
100.0 mg.	H	87.5	-12.5%	Average	Slight agitation*
100.0 mg.	I	87.0	-13.0%	Average	Slight agitation*
200.0 mg.	J	166.0	-17.0%	Average	Slightly more agitation*
75.0 mg.	K	74.0	- 1.0%	Large	Normal routine care
75.0 mg.	L	75.0	0.0%	Large	Normal routine care
100.0 mg.	M	85.0	-15.0%	Very narrow	Normal routine care
50.0 mg.	N	46.0	- 8.0%	Narrow	Normal routine care
100.0 mg.	O	75.0	-25.0%	Narrow	Slight agitation*
URIC ACID					
4.00 mg.	P	3.1	-22.5%	Reagent of Folin's modified method. (About 1 year old)	Folin's normal routine for this method
4.00 mg.	Q	3.6	-10.0%		
4.80 mg.	R	4.2	-12.0%		
6.40 mg.	S	5.3	-17.2%		
8.00 mg.	T	6.6	-17.5%		
			5)79.2		
			15.8%	is the average correction to be applied to all readings in this group	
UREA NITROGEN					
15.00 mg.	U	14.5	- 3.3%	Fresh Nessler's reagent prepared from old mercuric iodide stock sol.	Standard routine throughout
20.00 mg.	V	18.0	-10.0%		
40.00 mg.	W	37.0	- 7.5%		
40.00 mg.	X	35.0	-12.5%		
60.00 mg.	Y	58.0	- 3.3%		
60.00 mg.	Z	57.0	- 5.0%		
80.00 mg.	AA	71.0	-11.2%		
100.00 mg.	AB	83.0	-17.0%		
120.00 mg.	AC	95.0	-21.0%		
			9)90.8		
			10.1%	is the average correction for this group	

\*All cases of deliberate agitation were for a period not longer than two seconds.

The present reading technic, by means of the "Duboseq" type\* of colorimeter, is supposed to eliminate such variations in readings as are shown in the table, through simultaneous treatment of a standard with the unknown. Unfortunately, however, these errors are probably only rarely eliminated by running

\*The "Duboseq" type colorimeter optically compares the unknown solution with a standard, which has been similarly treated chemically.

a control simultaneously with the unknown inasmuch as certain factors are not routinely so exactly the same as it is supposed. The data presented will confirm this contention. For instance, in a blood sugar analysis, of all the tubes that are used (Standard and Control), the constricted neck in each will routinely vary considerably, thereby controlling the rate of diffusion of the phosphomolybdic acid with the initial contents (see "K," "L," "M," and "N" in the table). Also routinely one of the tubes may be shaken slightly more than the other after the reduction of the copper, causing a relatively large error for the amount of agitation, the effect of which is demonstrated by readings "H," "I," "J," and "O." The maximum agitation time was not more than two seconds in any case.

The relatively large errors which result from this agitation, makes it readily conceivable that sufficient shaking may occur routinely, to cause considerable errors in readings. In other words, if the unknown is disturbed more than the standard, the error will be negative. Whereas, if the standard is disturbed the more, the error will be positive. Or if both should receive precisely the same amount of agitation, the errors (negative and positive) due to this one factor would cancel out. This factor is so critical that the latter case rarely occurs in routine work.

When we consider the urea nitrogen analyses by Nessler, we see a colorimetric test that has been classic in being both critical and erratic, but nevertheless valuable when its shortcomings are accounted for. In these analyses there is often a distinctly different reaction involved in the unknown, relative to the standard. For instance, very small differences in the acid base equilibrium as are commonly introduced in the unknown relative to the standard will bring about a decided cloudy or imperfect color reaction in that unknown. At any rate the reaction is definitely of a different character than occurs in the standard with which it is compared. The urea nitrogen group in the table reveals the magnitude of the erroneous nature of the reaction of Nessler's reagent with even the C. P. ammonium sulphate solution that was used throughout this series.

In Folin's modified method for the analysis of uric acid, the age of the reagent is the important factor affecting the specific color density. The uric acid series in the table demonstrates the diminution in specific color density to be expected from this factor. The 15.8 per cent represents the average approximate diminution for a one year old reagent. (All other solutions used in conjunction with this series were fresh and standardized.) The marked deviation in the individual errors from the average (15.8 per cent) probably represents the effect of uncontrollable factors, which undoubtedly introduce erratically errors of these general magnitudes to routine tests.

It has apparently been the custom of clinical laboratories to overlook the grossness of errors introduced through these hidden factors and profess a precision of readings of the unknown of 1 per cent or better, in all these tests. Although one does not actually vouch for the accuracy of a reading, one suggests the precision of the reading by the number of significant figures which he writes in the final result. For instance, if he reports a normal blood sugar analysis to 0.1 mg, he is suggesting an overall precision within  $\frac{1}{10}$  per cent. In view

of the foregoing data, figures to the right of the decimal point in readings of approximately 100 mg. or over are certainly superfluous and meaningless. In any case where a certain digit is superfluous, mathematicians have taught us to substitute a zero in its place, if it lies to the left of the decimal point, and to drop the digit entirely if it lies to the right of the point; and retain a numerical value for only those figures which are really significant. Certainly no more than three digits should ever be used to represent the results of a routine colorimetric test. The author believes that it is only jeopardizing incentive for research and ultimate improvement in colorimetric chemical methods to profess a precision within 1 per cent or better in the majority of clinical colorimetric tests done by the present methods.

The authors' investigation of the degree of precision of colorimetric reactions was aided by a unique photo-electric colorimeter, which was developed after

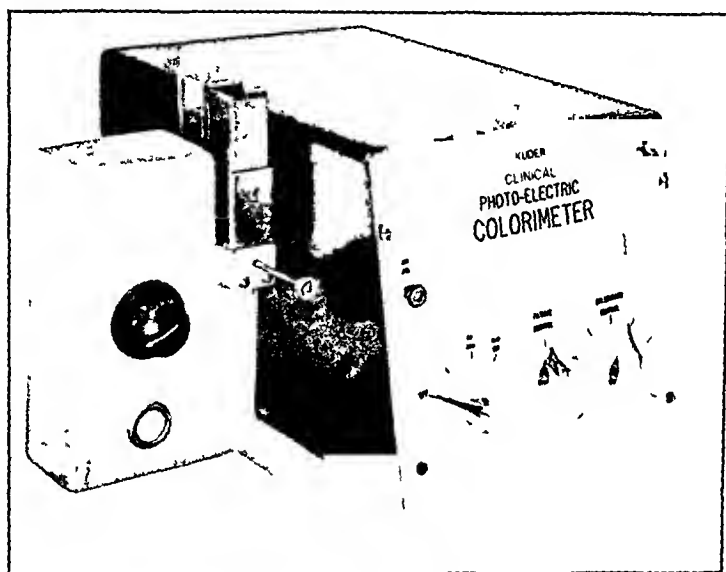


Fig. 1.

a year's research and careful study of desirable color reading devices. Fig. 1 shows a front view of this instrument which embodies the following desirable features:

First, a multiple set of direct reading scales (see Fig. 3) are provided on a drum behind the slot in the front panel which are calibrated directly against many successive weighed increments of the respective chemicals. This permits the reading of the unknown directly in the desired units without any necessity of intercomparison against a standard solution. (Fig. 2 shows this mechanical structure.) The discussion near the end of this article attempts to confirm the desirability of this altered procedure.

Second, this instrument has the ability to read a specific color density directly and easily within 2 per cent accuracy. There has been substantial evidence of this accuracy through periodic checks against several permanent dry color standards, which indicate no appreciable drift in the calibration in a year.

Third, the unique photo-electric indicator circuit offers a simple means of controlling any possible drift in the initial calibrations after long service.

Fourth, a set of dry color standards of good stability incorporated in the unit may be used conveniently to check the calibration periodically. These color panels are introduced into the light path by means of the little button at the left side of the front panel labeled "Dry Std." (see Fig. 1) which operates a sliding mechanism carrying a set of filters.

Fifth, the electric eye offers an indisputably more precise means of measuring light intensity than the human eye, and in this application it could eliminate

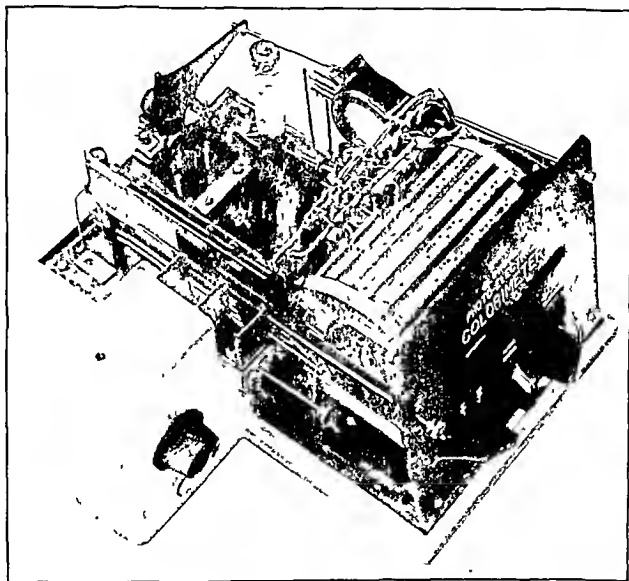


Fig. 2

another one of those undesirable human elements from a routine scientific measurement.

The fundamental analysis of the present system of the matching of colors in a "Duboseq" type colorimeter resolves itself into an attempt to match the intensity of the stimuli to the brain resulting from the separate excitation of opposite sides of the retina. An analogy of this type of measurement is the determination of the weight of an object by holding it in an extended hand and attempting to adjust a standard weight in the other extended hand which will equal the unknown weight, by comparison of the muscle reaction of each arm. In both the case of the retina and the extended hand, differences in such factors

as initial development, fatigue, etc., greatly influence the accuracy of measurements undertaken with these organs.

The authors anticipate the reluctance of chemists and technicians to abandon the control or standard in these tests. They agree, however, that the control should not be abandoned completely but suggest that with this type of colorimeter the control would be needed only periodically for many routine tests. If we consider for a moment the fundamental purpose of treating a standard solution simultaneously with the unknown, as is the practice, we find that this dual chemical treatment, if it is perfectly similar to the superlative degree, eliminates errors in the reading of the unknown which might otherwise be introduced through variations in treating technique, such as variations in temperature, time, concentration or intrinsic activity of reagents, etc. However, in view

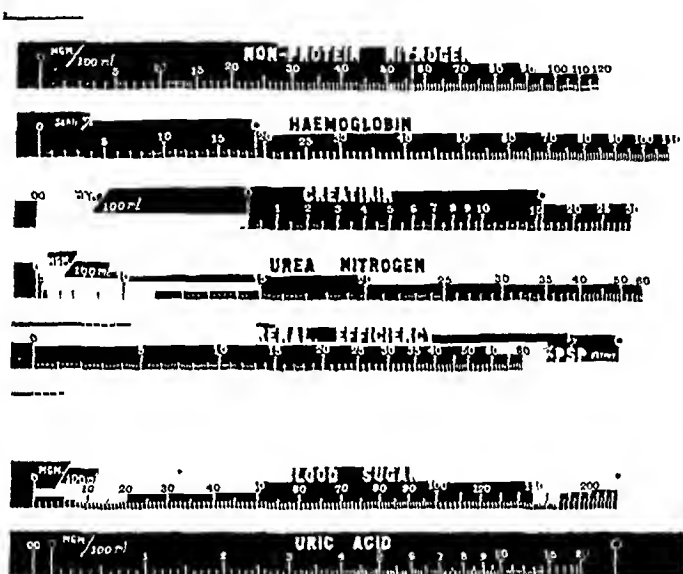


FIG. 3.

of the foregoing discussion of the data of Table I, it should be obvious that the absolute color density of both the standard and unknown solutions, when treated with routine (not absolute) similarity, will usually vary, irrespectively, about 10 per cent above or below the par\* color density. Furthermore, if this variance is of the same sign and percentage of magnitude, in both the standard and the unknown, it is certainly a purely accidental coincidence, as it was shown that this variance does not routinely have as close a relation to the treating technique as is generally supposed. Obviously the control for *each* routine test has more often played the rôle of a necessary adjunct to the present comparison colorimeters, rather than being the proverbial guardian of precision.

Since the sign and the magnitude of the variances (from par color density) in the standard and unknown solutions are only remotely correlated routinely, the authors suggest that a reading of the absolute color density of the unknown,

\*Par color density is that density which theoretically should be obtained with perfectly standard chemical treatment throughout.

directly in terms of the desired units, yields a result fully as reliable as does the present routine method, and in many cases more accurate and by reading a routinely prepared standard periodically, a simple percentage correction can be applied to the readings of routine analyses to compensate for deterioration of the reagent. With this correction applied the results, which this direct reading type instrument yields, are definitely more accurate. If this direct reading method were ultimately adopted, the resulting elimination of the control for each test of many types would greatly simplify the routine clinical work in colorimetry.

The authors offer their colorimeter design as the possible adjunct to this suggested modernization which they believe will eventually be universally adopted for routine clinical work.

#### SUMMARY

- 1 Some unrecognized errors in present methods of colorimetric determinations have been noted and explained.
- 2 A new type of photo electric colorimeter has been described.
- 3 A modernization in colorimetric procedure is suggested, which offers simplicity and accuracy.

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### IODIZED OIL\*

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#### A PRACTICAL METHOD OF PREPARATION

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FOR many years iodine derivatives of the fatty oils have been known to chemists, but the diagnostic and therapeutic value of such preparations was not generally recognized by the medical profession until about 1921.

According to Siead and Forestier,<sup>1</sup> the first true iodized oil was prepared in 1856, by Personne, who introduced a small amount of the element into cod liver oil. In 1896, Wintermütz produced a chloro iodized sesame oil known as iodipin, though he gives Hubl<sup>2</sup> (1884) credit as the first to devise a practical and reliable technique for this procedure. In 1901, a good iodized oil, containing 40 per cent iodine but no other halogen, was made by Lafay.<sup>3</sup> This oil is known as lipiodol.

During the last fourteen years, in response to the increasing demand for a suitable iodized oil, numerous methods of preparation have been advocated. In most of the procedures, iodine is made to combine, at the double bond, with unsaturated fatty acid radicals through the intermediate formation of iodine chloride, which in turn liberates hypiodous acid<sup>4</sup> by a secondary reaction with water. Whether the iodine halide is prepared by decomposition of iodine trichloride, chlorination of iodine in solution by means of chlorine gas<sup>5</sup> or aqua regia,<sup>6</sup> or with the aid of mercuric chloride,<sup>7</sup> the resulting action upon the oil

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is essentially the same. Iodine and its companion halogen are fixed at the unsaturated valences of the double linkage in a ratio that is, in most cases, one to one. Lipiodol is supposed to be made by the action of hydriodic acid on poppy-seed oil. The reaction is undoubtedly similar to that which occurs when crueic acid is converted into monoiodobehenic acid.<sup>8</sup>

Lipiodol is a very satisfactory oil for diagnostic and therapeutic purposes but is expensive and comparatively unstable when exposed to air and light. The iodized rape seed oil (capiodol)<sup>7</sup> is reported to deteriorate only very slowly, but the process of manufacture is somewhat too tedious for those physicians who desire to prepare their own product. Sesame oil, iodized according to the method of Schon and Jacobsen,<sup>5</sup> is said to be unaffected by air, even after exposure for long periods, and the technic is relatively simple though time consuming, because the final product must be aerated for twenty-four hours before it is ready for use.

We are now making an iodized oil in our own laboratory by a convenient and practical method in which iodine trichloride is first employed with the subsequent production of iodine monochloride (iodine chloride) as the intermediate iodizing agent. This procedure was originally reported in foreign literature. We are greatly indebted to Major R. L. Holt, of the Fitzsimons Army Hospital, Denver, Colorado, for first calling our attention to it and for many helpful suggestions concerning other steps in the preparation of the oil.

#### TECHNIC FOR PREPARING AN IODIZED POPPY-SEED OIL

*I. Preparation of Iodine Trichloride.*—Place 35 gm. of resublimed iodine crystals in a large, dry test tube immersed for three-fourths its length in water at 15° C. Introduce the free end of a glass tube (leading from the flow meter of a Dakin chlorinating apparatus) clear to the bottom of the iodine container in such a way that the iodine crystals lie loosely around it. Allow the chlorine to flow into the iodine for twenty minutes at a "flow-meter pressure" not to exceed 45 mm. of water. The crystals are rapidly converted into a dark red liquid. As the reaction nears completion, the citron-yellow crystals of iodine trichloride ascend the walls of the test tube. At this point, ice is placed in the cooling bath.

*II. Decomposition of Iodine Trichloride With the Formation of Iodine Chloride and Hypoiodous Acid.*—To the iodine trichloride add 150 c.c. of distilled water at 15° C. and transfer it to a two-liter flask partially immersed in water at 5° C. As soon as the solution is complete (not more than ten minutes), 100 c.c. of cool, fresh poppy-seed oil are slowly added. Agitate during the introduction of the oil, and continue until all of the iodine chloride has been taken up by the oil. The finished product is a thick, ivory-white emulsion. Allow the preparation to stand for at least two hours.

*III. Separation of the Iodized Oil From the Acid-Aqueous Solution.*—Pour in 100 c.c. of pure chloroform, shake the new emulsion thoroughly for at least five minutes, and transfer the entire contents of the flask to a 1,000 c.c. separatory funnel. Stratification of the oil and aqueous layers starts rapidly, becoming complete in approximately three hours, though it is perhaps better to wait for six hours.

*IV Removal of Chloroform From the Iodized Oil*—Separate carefully the lower (oil chloroform) layer from the upper strongly acid, watery layer, which still contains some iodine chloride, place in a 2,000 cc evaporating dish which is heated by a boiling water bath. Within twenty minutes the temperature of the contents of the evaporating dish should be 80° C, at which temperature chloroform is rapidly expelled. At the end of thirty minutes, place an electric fan so that a current of air sweeps continuously across the surface of the liquid, and continue heating, with frequent stirring, for an additional two and one half hours. The chloroform is eliminated much more rapidly if air is bubbled through the mixture during the entire process of heating.

The appearance of the oil changes several times. For about ten minutes it is a creamy tan while the major portion of the chloroform boils away. At the end of thirty minutes, the color has changed from a pinkish brown to a mahogany brown, then to a pale yellow. During the next two hours, the color becomes and remains a pale amber, the darkening being due to a slight amount of oxidation.

Removal of the chloroform is complete only when the taste of this chemical is absent even when the warm oil is dropped on the side of the tongue.

While still warm, the oil is passed through fine muslin and then through dry filter paper to remove a small amount of resinous material (oxidation products), transferred to 20 cc containers which are filled to the brim and covered by an aluminum cap.

Before releasing the oil for therapeutic purposes, each lot is checked by shaking separate portions with water and with a potassium iodide solution and testing the aqueous phase of the first mixture with litmus (free acid), and of the second with starch paste solution (free iodine). No free acid or iodine should be found.

*V The Finished Product*—Prepared by the foregoing method, the iodized oil is a moderately viscous, pale amber, neutral liquid with an oleaginous taste (resembling "cooked" oil) and a specific gravity of 1.20 to 1.28 at 37° C. Its viscosity, compared with water at 37° C, is from 380 to 410 or 260 to 280 centipoises. It has an iodine content of 22 to 25 per cent and a chlorine content of 6 to 8 per cent. The viscosity, gravity, iodine and chlorine content vary with different batches. It contains no free iodine. Little or no color change, and neither free acid nor free iodine can be detected after standing for several weeks in the dark. Slight darkening is observed when the product is exposed for the same length of time to light but repeated tests still fail to show the presence of free iodine or free chlorine.

As previously stated, the oil contains chlorine, however, its presence is in no way objectionable clinically. On the other hand, we have evidence to indicate that the presence of chlorine actually lends increased stability.

Recently we<sup>9</sup> have described somewhat in detail the clinical use of the oil.

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## A METHOD OF ELIMINATING BLASTOCYSTIS HOMINIS FROM CULTURES OF ENTAMOEBA HISTOLYTICA\*

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THE presence of *Blastocystis hominis* in cultures of *Entamoeba histolytica* interferes greatly with their growth and reproduction. In many instances this organism will alter the nutritional, chemical, and physical state of the culture medium so rapidly that *E. histolytica* can no longer survive. This, perhaps, is one of the most frequent causes of failure to maintain *E. histolytica* in culture.

Various workers in the field of protozoology have recommended the addition of a small quantity of heat-sterilized, dry rice starch to each culture, at the time of inoculation or transfer, to militate against the occurrence, or to control the growth of *Blastocystis hominis*. In the experience of the author this procedure does not rid the cultures of *Blastocystis hominis*, nor does it sufficiently restrict the growth of this organism to the point that it will not interfere with the growth of the amoeba.

Studies on the growth habits of *Blastocystis hominis* show that a pH of 7.4 to 7.8 affords the optimum reaction for its growth, while a maintained pH of 6.8 to 7.0 tends to inhibit its growth. In cultures where the bacterial flora splits the rice starch and the pH is reduced to 6.8 or 7.0, the growth of *Blastocystis hominis* is partially restricted; in cultures containing rice starch where this reduction in pH does not take place, there is no inhibition of its growth.

The influence of various concentrations of neutral acriflavine upon cultures of *E. histolytica* contaminated with *Blastocystis hominis* has been investigated with the following results:

1. *E. histolytica* will live and reproduce in a 1 to 50,000 solution of neutral acriflavine in suitable culture medium.

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2 Such a solution of neutral acriflavine in Boeck Drbohlav culture medium is toxic for *Blastocystis hominis*, and tends to inhibit its growth and reproduction

3 If, after treating the cultures on Boeck Drbohlav medium with 1 to 50,000 neutral acriflavine for forty eight hours, three successive transfers at twenty-four hour intervals are made in suitable media in which the pH is maintained at 6.9 to 7.1, then transferred to regular Boeck Drbohlav medium, the *Blastocystis hominis* will completely disappear from the culture after several transfers and *E. histolytica* will grow unhampered

In accordance with the above findings, the following method has been devised which has never failed to rid *E. histolytica* cultures of *Blastocystis hominis*. At the time of inoculation, or transfer of all cultures in the subsequent procedure, 0.05 cc of a dry, sterile mixture of two parts of powdered rice starch and one part of powdered animal charcoal is added to each culture

1 Amoebae cultures containing *Blastocystis hominis* are treated as follows. Add sufficient sterile 1 to 10,000 neutral acriflavine in Ringer's solution

Ringer's Solution	{	Sodium chloride	8.0 gm
		Potassium chloride	0.2 gm
		Calcium chloride	0.2 gm
		Distilled water	1000.0 cc

to the supernatant fluid of the culture on Boeck Drbohlav medium to make a 1 to 50,000 solution. Incubate at 37° C for twenty four hours, transfer the culture, repeat the above procedure for another twenty four hours, and then transfer the culture to lactic acid medium prepared as follows

2 *Lactic Acid Medium*—Prepare the following modified Locke's solution and sterilize it in an autoclave at 15 pounds' pressure for fifteen minutes

Locke's Solution	{	Sodium chloride	9.00 gm
		Calcium chloride	0.24 gm
		Potassium chloride	0.42 gm
		Sodium bicarbonate	0.20 gm
		Lactic acid, 1.0 N	0.23 cc
		Distilled water q s ad	1000.00 cc

With sterile precautions, mix one part of sterile horse serum with seven parts of the cool, sterile Locke's solution. Then add 5 cc of the resulting solution to previously prepared sterile egg slants, i.e., the solid media prepared for Boeck Drbohlav media. Transfers of the cultures on lactic acid medium are made at twenty four hour intervals for three successive times and then the cultures are transferred to straight Boeck Drbohlav medium. There will still be some *Blastocystis hominis* present in the culture, but the organisms will usually all be in a degenerated condition and will completely disappear upon successive transfers of the culture. In case they do not, the entire procedure is repeated, and to date, the second repetition has never failed to eliminate this organism

# A METHOD OF ESTIMATING BOTH BASAL AND EXERCISE CARDIAC OUTPUT ON DOGS\*

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IN VARIOUS laboratory studies it would be advantageous to know an animal's cardiac output during exercise as well as at rest. Such data, if reliable, would be useful in experiments involving changes or attempted changes in the cardiovascular system or in estimating physiologic reactions to exercise before and after experimental interference with the animal. Though basal, or resting, cardiac output is readily estimated in the dog by application of the Fick principle, a measure, by similar means, of the exercise cardiac output meets with several obstacles. One must not only find out what the oxygen consumption per minute is while the dog is actually at work but must also obtain samples of blood representing the arterial and venous oxygen content during work. In order that comparisons can be made between various experiments the amount of work done must be ascertainable. Since no standardized method is available the method we use with satisfaction is presented. The usual procedure will be given briefly and then amplified and discussed fully under appropriate headings.

## METHOD

Male dogs, found satisfactory by trial for the work, are standardized regarding heart rate, respiratory rate, temperature, blood pressure, and resting cardiac output. The blood pressure is taken by direct femoral artery puncture. The resting cardiac output is obtained by application of the Fick principle. The oxygen consumption is measured by use of a Blalock mask and a closed circuit spirometer with valves. The oxygen content of the right and left heart blood (or femoral artery blood) is measured on a Van Slyke machine.<sup>1, 2, 3, 4</sup>

While the dogs are being thus standardized, they are run on an electrically driven treadmill a little each day until they are accustomed to it. At the same time a special mask, described later in the text, is little by little, on successive trials, fitted over the dog's head until he tolerates it satisfactorily. The dog is then, in a similar manner, accustomed to breathing into an attached closed circuit spirometer while he runs.

The dog having been thus prepared, the actual estimation of exercise cardiac output is done by running the dog about a half hour, at a certain speed, and with the floor of the mill at a certain slope, during which time several oxygen consumption curves are taken over five- to ten-minute periods. At the end of half an hour the dog is rapidly removed from the mill and arterial and right

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heart blood samples are taken. One pair of samples are taken within one half minute of cessation of exercise usually and two other pairs at intervals of about one minute each. As the first two pairs of samples usually agree closely it can be assumed usually that the first arterial and venous samples represent, with fair accuracy, the arterial and venous oxygen values when the animal is at work. By use of the Fick principle the exercise cardiac output is calculated from these figures. A check of the approximation of the oxygen content of such blood samples to the actual arterial and venous oxygen content at work is carried out when all studies on a dog are completed. Samples of right and left heart blood are taken during actual exercise, both while oxygen consumption is being measured, and while the mask is not worn. A close agreement is found to exist between the figures obtained by the two methods.

#### DISCUSSION OF METHOD

*Preparation of Animals*—It is worth while to choose carefully the animals used in these experiments. Male dogs only can be used because the females have an unstable cardiac output. A dog anxious for work, intelligent and yet of a passive disposition, proves excellent. By a brief trial consisting of several fittings of the Blalock mask and the special mask, a few runs on the treadmill, and several trials at resting oxygen consumption, it can be readily judged whether a dog is likely to be satisfactory. Suitable dogs are accustomed to lying quietly in the dorsal position on an animal board, to the Blalock mask connected with the spirometer, to cardiac and arterial puncture, and to running on the treadmill. When the dogs are quite used to the treadmill the special mask is put on little by little, on successive trials, until the animal runs quite nonchalantly with the mask completely adjusted. Because it is found that often, on the first few occasions that the mask is connected with the spirometer, the dog is slightly disturbed, gradual initiation in this maneuver results in the dog rapidly becoming used to the procedure.

*Basal Output*—The basal cardiac output is calculated in the usual way except that we prefer to take samples of right and left heart blood (or femoral artery blood) several times during the course of one of several resting oxygen consumption estimations. An accurate judgment can thus be made of the reliability of the test. To check our results further the circulation rate is tested from time to time, during a basal oxygen consumption test, by the cyanide method. This, with a count of the pulse and respiratory rate, forms a useful guide to the animal's true state of rest.

*Exercise Output*—To explain more fully the rationale of the method which we feel is satisfactory for obtaining a figure accurately representing the cardiac output in exercise, the method will be discussed and amplified under the headings: The Oxygen Consumption Estimation, Estimation of the Arteriovenous Oxygen Difference, and Estimation of Work Done.

*The Oxygen Consumption Estimation*—An estimate of the oxygen consumption of exercising dogs has been attempted by some by taking the oxygen consumption measurement immediately after the cessation of work. A variety of masks have been used, some of which fitted the snout tightly like the Blalock

mask, while others fitted over the head. The former type will never fit a dog satisfactorily that is breathing in a manner corresponding to its exercise respiratory volume. In any event, no matter what mask be used, the method can be shown to be inaccurate. Certain dogs maintain a high oxygen consumption for some minutes after exercise with long continued excessive ventilation and

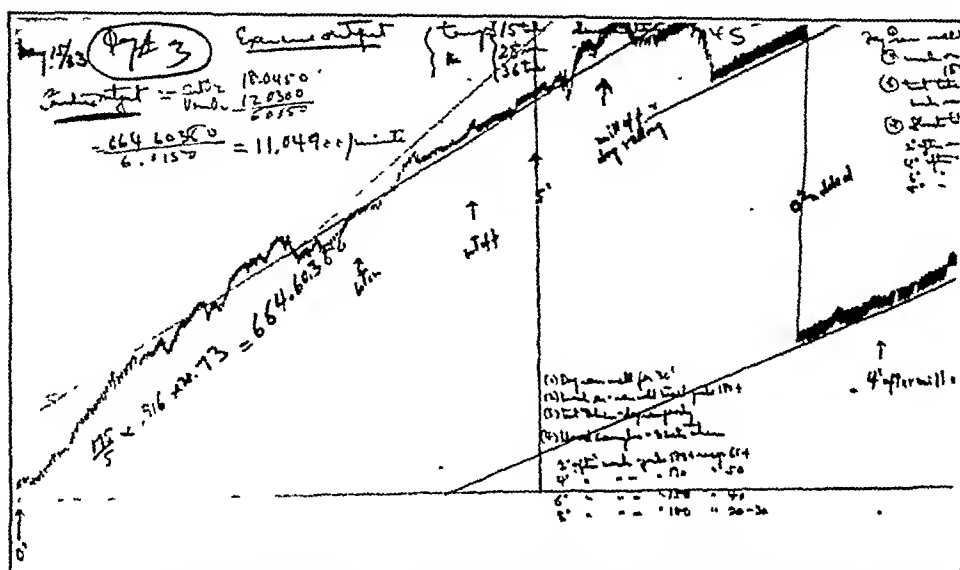


Fig. 1.—The oxygen intake curve of a very active dog. Its oxygen intake remained high for some minutes after the treadmill was stopped and the dog was made as comfortable as possible.

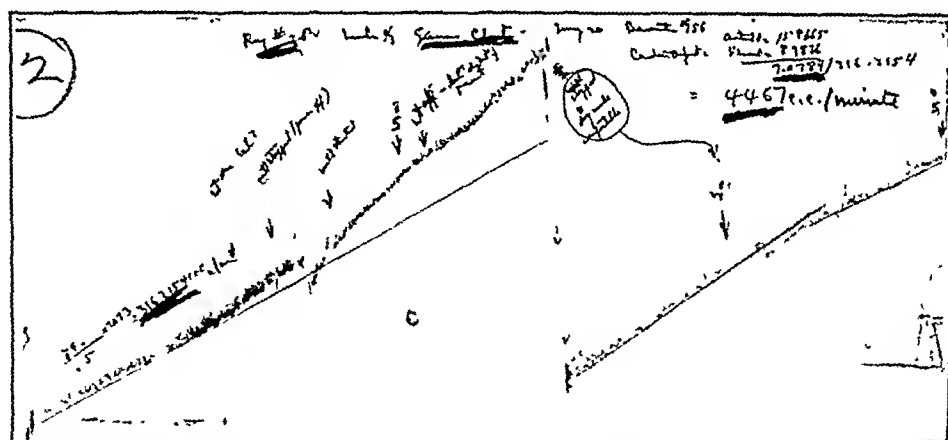


Fig. 2.—The oxygen intake curve remained high for this dog after cessation of work but the arteriovenous oxygen difference increased. This lowered the figure for exercise cardiac output to 4467 c.c. per minute. The exercise cardiac output was usually about 10,000 c.c. per minute when oxygen intake and blood sample were taken while the animal was actually running.

panting (Fig. 1). An oxygen consumption curve, taken with a suitable mask on such dogs after work, would sometimes be useful but would not represent reliably the actual exercising oxygen intake figure. Moreover, though oxygen intake on such a dog remains near the exercise level after work for some time, the venous oxygen content may change rapidly because of slowed circula-

tion and, by altering the arteriovenous oxygen difference from its exercise level, may produce a change in the figure for the cardiac output. Fig. 2 illustrates an instance where oxygen intake remained high for some minutes after cessation of work but the venous oxygen content fell rapidly to increase the arteriovenous oxygen difference and diminish the figure for cardiac output. On the other hand, many dogs exhibit an exercising oxygen consumption curve which very rapidly falls toward the basal level after exercise. These dogs can usually be fitted with a mask that fits either the snout or the head tightly, for they pant very little. But the curve obtained in this way is of negligible value. Fig. 3 illustrates how an oxygen consumption of 282 c.e. per minute during

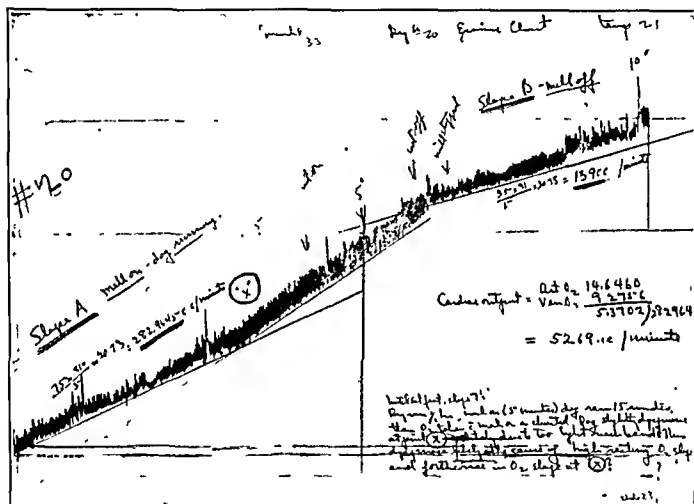


Fig. 3.—The first six minutes of oxygen intake. Slope marked "Slope A" is taken with the dog running. "Slope B" is much lower and commences when the dog stops running.

exercise, represented by the first six minutes of the curve and marked "slope A," changed rapidly on cessation of work to an oxygen consumption of only 139 c.e. per minute, represented by the last four minutes of the curve and marked "slope B." Fig 4 illustrates a typical exercise consumption curve taken on a dog actually at work and the oxygen consumption curve on the same animal taken immediately after the cessation of work. The former curve represents an oxygen consumption of 337 c.e. per minute while the latter represents an oxygen consumption of 97 c.e. per minute.

Therefore it seems more accurate to measure the oxygen consumption while the animal is actually at work. Intertracheal cannulas may of course be used but are liable to cause the animal discomfort requiring much time and in many



dogs during exercise may leak or become plugged by an overproduction of mucus. For these reasons several masks which a dog could wear while running were designed. Lightness, visibility, ease of adjustment, and air tightness were the prime requisites. The mask which proved most suitable is readily and very cheaply made as follows:

A celluloid cylinder is rolled of suitable dimensions just to fit over the dog's head. Ordinary celluloid or cleaned x-ray film may be used, the edges be-

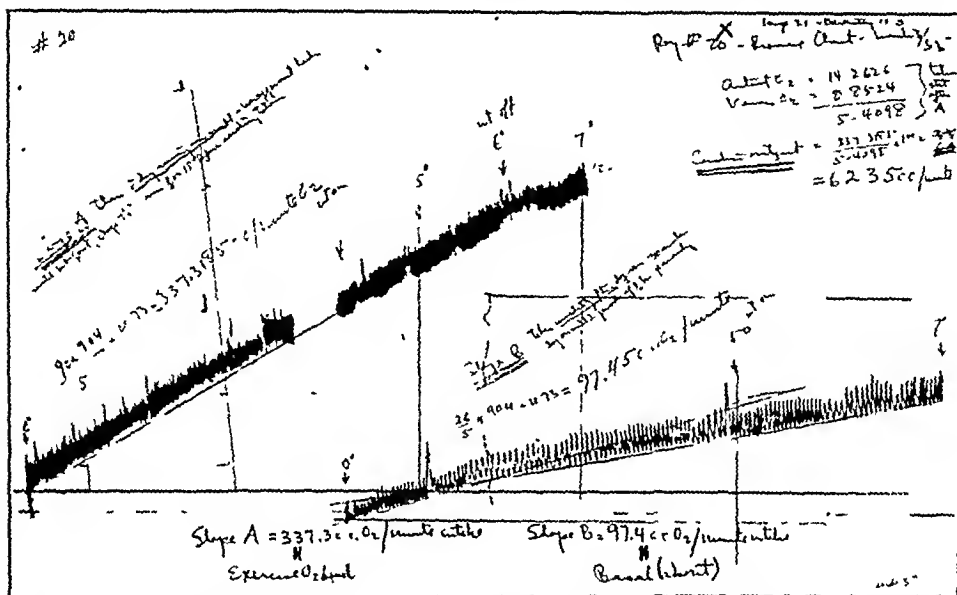


Fig. 4.—"Slope A" illustrates the high oxygen intake while a dog runs. "Slope B" illustrates a very much lower oxygen intake immediately after exercise was finished.

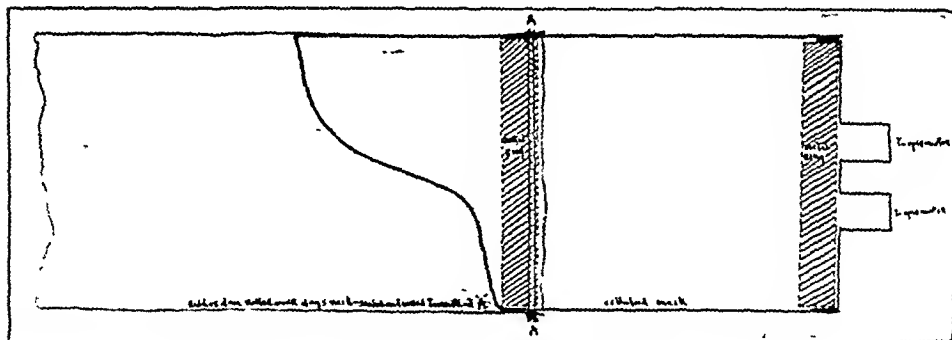


Fig. 5.—Diagram of mask.

ing easily sealed with acetone or by the application of an adhesive such as "Ambroid." The cylinder's length is such that with its one end several inches in front of the snout, the other end comes just behind the ears. A light metal ring supports the front of the cylinder while toward the back another ring lends support and also has a cord fixed to it which can be fastened behind the dog's neck to hold the mask in place. The front of the cylinder is fitted air tight with either a light tin or celluloid disc equipped with two centrally placed

tubes designed to fit exactly the ends of the spirometer tubes. The caudal end of the cylinder is shaped in its upper half to project a little over the back of the dog's neck while the under half is cut in a curve to fit the dog's throat. A piece of light rubber dam made in tubular fashion is tightly bound and sealed to the caudal end of the mask and can be rolled over the dog's shaven neck which it fits snugly. It is further held to the neck by several wide rubber bands carefully adjusted to avoid undue pressure, especially on the veins (Fig. 5).

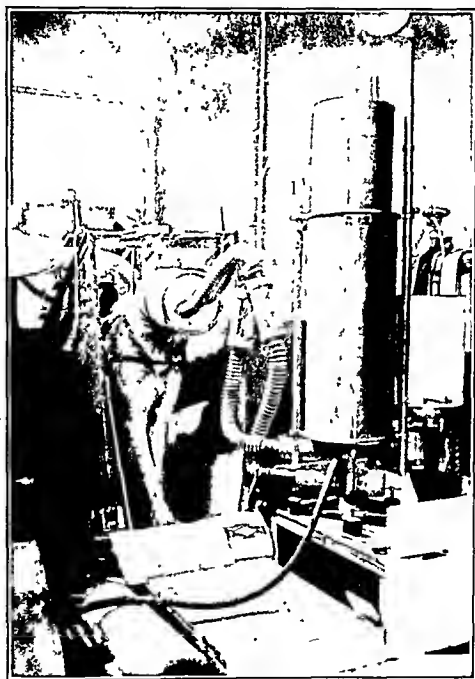


Fig 6—Photograph of dog on treadmill wearing mask which is joined by flexible tubing containing flutter valves in celluloid housings to a closed circuit type spirometer

Such a mask has proved very satisfactory and surprisingly leak proof in our hands. Each experiment is tested for leakage. Small leaks must in any event prove negligible on account of the very large oxygen intakes registered. Dead space in the mask is also negligible for the same reason and because the dog's head leaves little space actually unoccupied. In warm weather humidity in the closed circuit used occasionally makes the animals uncomfortable. Removal of the apparatus to a cold room corrects this difficulty.



found that if three sets of femoral artery and right heart blood samples are taken immediately work is finished, at intervals of about a minute, an idea can be had of how closely the first set of samples approximates the actual exercising arterial and mixed venous oxygen figures. Accordingly the following technique is followed. A sample of femoral artery and of right heart blood is usually easily obtained within the first half minute of stopping exercise, the dog being rapidly removed from the treadmill and placed in the dorsal position on an animal board. This first pair of samples usually indicates an arteriovenous difference of 3 or 4 cc of oxygen per 100 cc. The venous oxygen content usually falls a little in the second pair of samples to give a slightly increased arteriovenous difference. The third pair of samples, often taken two or three minutes after work has ceased, usually gives a much greater arteriovenous difference due to lowered venous oxygen content. There is presumably, then, no great change between the arteriovenous oxygen difference during exercise and during the first minute of rest following exercise. By this procedure, however, the animal is put suddenly at comparative rest and simultaneously removed from an atmosphere of variable oxygen tension, but still one which might be of higher oxygen tension than that of atmospheric air. Since both these factors are possibly capable of changing the arterial and venous oxygen relationship a check of the results is made on each dog when its experimental usefulness is completed, and death caused by accidental tamponade is of less consequence. Samples of right and left heart blood are taken while the dog is running and the oxygen intake is being measured and while the dog is running free of the mask. The figures for the arteriovenous oxygen difference obtained in this way are found to agree closely with an average of the figures for the arteriovenous oxygen difference of the first arterial and venous samples drawn from the same dog immediately after exercise.

It is found that each dog requires a few tests before an idea can be had of its usual reaction to exercise as seen in arterial and venous oxygen estimations. In this series of experiments, in the majority of dogs during moderate exercise, the venous oxygen content rises somewhat, the arterial oxygen content changing either way only slightly. Individual dogs vary to only a small extent from time to time in their arteriovenous blood oxygen difference during exercise though occasionally larger variations do occur (Figs 8 and 9). Such changes seem due to alterations in circulation time. Because of such variations, and because the rise in the venous oxygen content during exercise in the majority of the dogs is apparently no artefact, circulation time is frequently taken by the cyanide method. As a rule dogs that show a smaller arteriovenous oxygen difference during work than at rest have a very short circulation time while exercising. Dogs that during exercise show an increased arteriovenous oxygen difference over that at rest have a long circulation time at work. This small arteriovenous oxygen difference in exercise seems due to such an increase in circulatory rate in response to exercise that, though oxygen utilization in the tissues increases, the relationship between circulation rate and oxygen utilization is such that venous blood oxygen is not usually reduced but is frequently

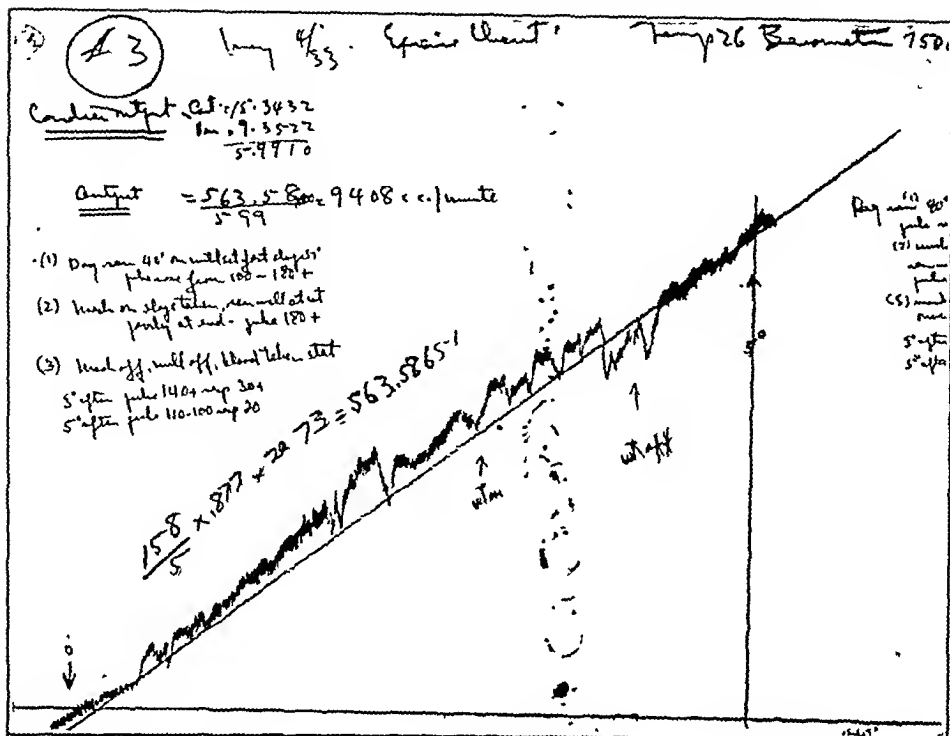


Fig. 8.

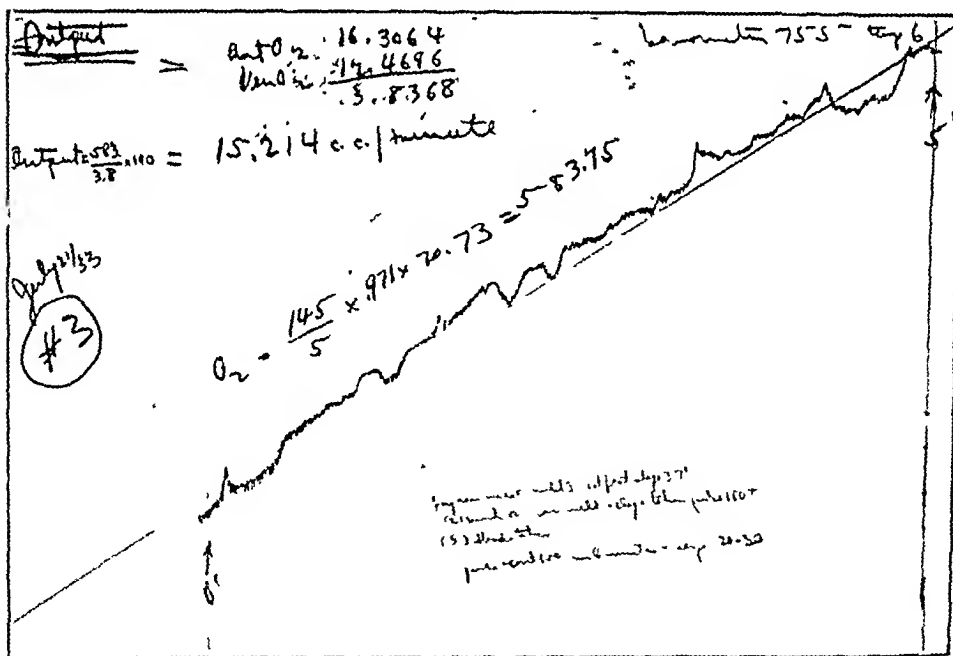


Fig. 9.

Figs. 8 and 9.—Dog 3, May 4, 1933, used 563 c.c. oxygen per minute but because the arteriovenous oxygen difference was 6 c.c. per hundred the cardiac output was only 9,408 c.c. per minute. July 21, 1933, the oxygen intake was 583 c.c. per minute, the arteriovenous difference was 4 c.c. and the cardiac output was 15,214 c.c. per minute. A very similar amount of work was done on each occasion. Such variations occasionally occurred making it necessary to carefully study each dog's individual reaction to exercise so that the average reaction could be known and deviations recognized.



done on them and their cardiac responses compared. Severe exercise, though it gives an estimate of the maximum amount of work the dog is capable of, entails a training factor which is changed by periods of rest such as follow operations. The amount of exercise used in our particular experiments has proved severe enough in any event to give cardiac outputs four to six times the resting output, sufficiently high that any change in the ability of the heart to perform work is readily recognizable. This increase in output is also great enough to offset any of the small errors involved in the method. The time period is kept to one-half hour so that the effects of fairly hard work over a relatively short period are produced. Longer periods of time tend again to train a dog, thus changing from time to time the reaction to exercise of the various functions studied. Other factors in favor of moderate work are a fairly constant reaction from trial to trial, no great variation in temperature with accompanying metabolic changes and no undue disturbance to the animal.<sup>3, 5, 9, 11, 12</sup>

The treadmill used is electrically driven and is run at a speed of about 70 meters per minute with a slope of 37 degrees. The amount of work may be interpreted in terms of energy expended per kilogram by using the formula  $E = (0.6 \times a \times \cos 37^\circ) + (3 \times a \times \sin 37^\circ) = a \times 1.8$  where "a" represents the distance run at a 37° slope.<sup>11</sup>

There is a slight error in the calculation of work done because it is found safest to hold the dog in the mill with a leash. Though some dogs will usually run without a leash others tend to lag. The leash is fastened to a comfortable harness on the dog and to a suitable crossbar on the mill above and just ahead of the dog's resting position. Dogs that run well expend energy as governed by the speed and slope of the mill. But dogs that persistently have part of their weight carried by the leash are discarded. Careful note is kept during each experiment of the dog's behavior and performance of work, of the number of minutes taken for pulse, respiration, and blood pressure to return to normal levels, and of the dog's general appearance after work.

Though it is true that a dog's performance of work varies from time to time, for comparative purposes there is a close enough correspondence between the amounts of work done to justify comparisons between the cardiac responses of a dog from test to test. Tables I and II list the various data collected on two dogs of a series that were being studied to test the effects of coronary artery occlusion on dogs that had had previous stellate ganglionectomy. Table III represents the data on dogs that had not been subjected to stellate ganglionectomy before coronary artery ligation. The basal and exercising cardiac outputs of Tables I and II are fairly constant, with few wide variations, and support the reliability of this method of obtaining cardiac output at rest and during exercise. Further support is lent this view in that the cardiac outputs of all of the other group of dogs, represented by the data of Table III, show a fairly marked change after occlusion of the coronary artery. Apparently variations in cardiac response to exercise are easily discernible. The variation between the two groups of dogs noted in the altered cardiac output, both at rest and during exercise, is confirmed by clinical observation.

TABLE I

Typical figures obtained by use of the described method for basal and exercise cardiac output in a series of dogs that had stellate ganglionectomy done before coronary artery ligation was done. Clinically and by tests no great change in health occurred. Note the uniformity of the figures for output.

DOG NO	WT AML	PULSE RECOVERY REST WORK TIME	B T WORK R:ST	RESPIRATIONS RECOVERY REST WORK TIME	O <sub>2</sub> CONSUMPTION CO/MIN REST WORK	BLOOD O <sub>2</sub> ART VEN REST WORK	ART VEN O <sub>2</sub> DIFFERENCE REST WORK	ART O <sub>2</sub> C.P. REST WORK	CARDIAC OUTPUT REST WORK	E C G	HEART SIZE
60/33	130	100 150 2' 90 200 5'	85 115 90 120 80 121	16 28 3' 3 10 5	139 58 149 75 113 21	19 45 15 36 20 83 11 38 19 32 12 64	+1 9 5 69	20 2 237 218	5129 1584 1644	Dec 7	March 75 April 75
120	Stellate ganglionectomy		110 117 105 170 110 170		373 55 487 23 277 67	17 68 12 28 19 31 14 74 17 93 13 33	3 4 4 2 4 0		10 989 11 600 6 031		
152	80 150 6'		90 130 90 120 105 165 103 170	20 60 6'	164 0 108 0 183 3 142 0 116 0	22 0 16 4 16 5 13 1 22 1 16 3 22 7 17 9 18 2 11 0	6 5 3 5 5 8 4 6 6 3	24 6 26 5	2729 1144 3209 2709 1861	March 30	May 75
	Coronary ligation		90 120 90 130 105 175 110 170	30 60 5'	137 9 129 0 177 0 96 8	20 7 14 6 23 3 17 5 19 3 13 3 19 4 15 1 18 7 13 4 21 1 14 3	6 1 4 8 6 0 4 3 5 2 6 7	23 4 24 1	3259 2866 2932 2211 2596 2425	June 3	May 75 June 75 Aug 75
112	100 170 5'		90 120 90 130 105 175 110 170	30 60 5'	437 0 547 0 402 0	19 5 13 4 22 3 17 5 22 7 19 1	6 1 4 8 3 6		7 751 11 396 11 166		





TABLE III

Typical figures obtained by use of the described method for basal and exercise cardiac output in a series of dogs that had coronary artery ligation done without stellate ganglionectomy being performed. Clinically the dogs seemed less active after operation. Note the high basal output and reduced exercise output.

Dog No	WT KO	PULSE RECOVERY RFST WORK TIME	B P REST WORK	RESPIRATIONS RECOVERY RFST WORK TIME	O <sub>2</sub> CONSUMPTION CC/MIN RFST WORK	BLOOD O <sub>2</sub> ART VEN REST WORK	ART VEN O <sub>2</sub> DIFFERENCE REST WORK	ART O <sub>2</sub> C P	CARDIAC OUTPUT REST WORK	L C O	HEART SIZE
247	19.0	100 180 4'	80 150 75 140 75 145	25 60 7	147 141 118	220 184 236 171 235 167	30 65 68	24.5	3972 2169 1735	June 3	June 3 80
	17.0		98 170 95 168 98 170		330 579 434	183 134 208 160 173 134	4.9 4.8 3.9	24.0	6734 12020 11128		June 3 80
		Coronary ligature June 14, 1933									
	15.8	93 160 4'	78 140	25 60 4'	157.3 184.0 184.0	205 162 190 139 173 120	4' 5.1 5.3	22.8	3658 3607 3471		June 14 June 15 80
		90 150 4'	78 140		542 442 482 482	224 176 224 129 188 125 181 129	4.8 9.5 6.3 5.2		11291 4652 7650 9296		July 15 80

## SUMMARY

A method of estimating the basal and exercise cardiac outputs on dogs is given. A discussion of the various difficulties encountered and of the various means of checking estimations is gone into.

A mask we found altogether suitable for obtaining oxygen intake on dogs while at work is described.

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## AN AUTOMATICALLY RECORDING COLONY COUNTING APPARATUS\*

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AN INCREASE in the accuracy of bacterial plate counts can be brought about by eliminating the causes of physical and mental fatigue of the individuals making the counts. The strict attention required and the effort expended in counting and recording large numbers of bacterial colonies cause rapid tiring of the operator resulting in a decrease in his accuracy and productivity. The monotonous nature of the work is furthermore conducive to lapses of memory which not infrequently result in errors.

These drawbacks could be overcome and the speed of counting increased considerably were it possible to free the operator of the mental strain incident to counting the colonies. Attention could then be directed primarily to the detection of bacterial colonies rather than to their enumeration. In each of the counting devices of Stewart,<sup>1</sup> Buck and Swenarton,<sup>2</sup> Buck,<sup>3</sup> Schacht and Robertson,<sup>4</sup> Giau and others the objective has been primarily an increase in the definition of bacterial colonies by improving the lighting or magnifying systems. There is slight hope of further increasing the accuracy of plate counts by improving the methods for visualizing the bacterial colonies.

With the above factors in mind an apparatus has been devised by me for automatically recording the number of colonies in a Petri dish, and is described below. Following the completion of this equipment a search of the literature showed that Robinson<sup>5</sup> in 1930 had described an apparatus for this purpose, which consists of a marking pen so constructed that by pressing it against the glass back of a Petri dish, over a colony, a mark is left on the glass and an electrical contact is closed. This operates a magnetic counter which automatically records the number of the colony.

Artifacts in the medium or imperfections in the glass may interfere to a considerable extent with counting by such a method. Duplicate counts of certain colonies are not infrequently made, while still other colonies remain uncounted, especially when they are closely spaced or are growing in a thick layer of medium. Such a method furthermore renders difficult the use of counting charts. More accurate results should be obtained were the cover of the Petri dish removed and the count made by actually touching and simultaneously obliterating each individual colony. This procedure could be used since in milk and water work it is seldom necessary to study the bacterial colonies after they have been counted. Such a procedure would decrease the possibility of skipping some colonies and counting others more than once. Moreover, counts obtained by such a method could be readily checked since each colony actually counted would be plainly marked. The automatic colony

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counting device described below is so designed that each individual colony must be separately touched in order to be recorded.

The operating mechanism is enclosed in a substantial housing, through the metal top of which is cut a circular opening, slightly larger than a Petri dish, which serves to hold the latter. A removable cover glass is placed a short distance below this opening, and beneath this is placed a ruled glass counting plate which can be obliquely lighted from below by means of magnetically shielded electric lights so as to render visible the bacterial colonies. The metal top plate of the apparatus is connected through a high resistance to the 110 volt lighting circuit. If direct current is used, this plate must be connected

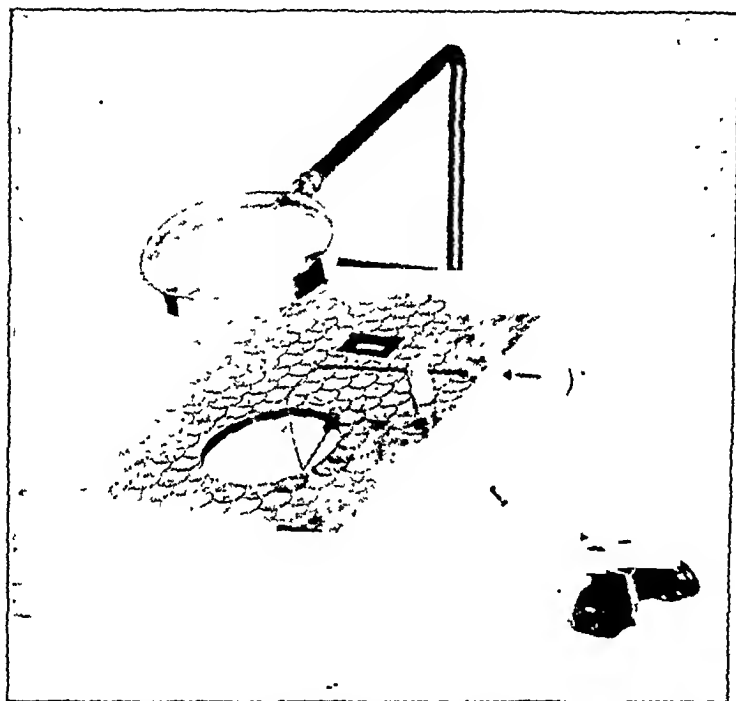


Fig. 1.—The recording colony counter, shown with the six inch magnifying glass in position.

to the positive input terminal only. The operator's hand must be in continuous contact with this plate while counting colonies.

An electrical connection is made between the agar or other nutrient medium and the recording mechanism by means of a removable clip which is connected through a high resistance to the grid of a vacuum or grid glow tube. The plate of this tube is connected to a sensitive relay which operates a magnetic counter. The magnetic relay can be dispensed with by substituting a Thyratron tube for the vacuum tube, but this is not recommended.

An electrical connection between the nutrient medium and the metal housing on which the hand of the operator rests is made by means of a metal electrode which is held in the hand of the operator. This electrode has an interchangeable tungsten tip, a blunt point being used for large colonies and

a sharp point for small colonies. It is readily manipulated since it is unattached to any part of the apparatus.

When a colony is touched the recording mechanism is operated by means of the imperceptible current which passes through the hand, electrode, agar, and high resistance to the grid of the vacuum tube. This minute current causes the grid to become positively charged, permitting the passage of a small plate current which operates the relay and trips the magnetic counter. When the electrode is withdrawn from the agar the grid again becomes negatively charged, stopping the flow of plate current and opening the relay. This resets the counter in readiness for the next operation.

The apparatus emits a distinct click each time a colony is touched, hence there is no danger of unknowingly counting a colony or of not knowing when a colony has been counted. Contacting a colony for one twentieth of a second suffices to operate the recording mechanism. If, due to unsteady

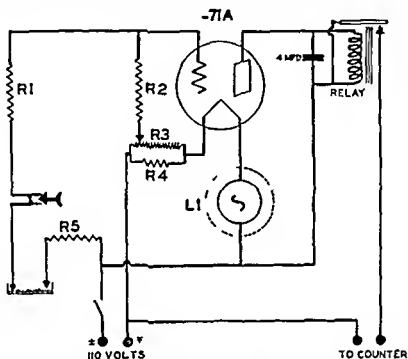


Fig. 2—Wiring diagram of the colony counter. R1-R5 overload protective resistances, R2 grid leak resistance, 113 grid potentiometer, R4 filament resistance, L1, 25 watt magnetically shielded show-case type light.

hands, a colony is accidentally contacted twice the resulting overcount can be compensated for by means of a circuit opening switch, the use of which permits the operator to touch the next colony without causing it to be recorded.

The apparatus is equipped with a large magnifying lens by means of which the bacterial colonies can be observed. There is adequate room between the Petri dish holder and the lens for the hands and counting electrode.

The apparatus is illustrated in Fig. 1 and the wiring diagram is shown in Fig. 2. The relay hook-up operates on both direct and alternating current, but the magnetic counter operates on only one type of current, which must be specified when securing this portion of the apparatus. The 4 mfd condenser shown is not needed if the apparatus is operated on direct current.

#### SUMMARY

A counting apparatus operated by a vacuum tube is described, by means of which bacterial colonies can be counted and recorded more rapidly and

accurately and with considerably less fatigue than by other methods. When a bacterial colony is touched with a free electrode held in the hand, a mechanism is operated which automatically records the number of the colony on a magnetic counter.

NOTE: This apparatus may be obtained from the A. S. Aloe Co., 1819 Olive St., St. Louis, Mo.

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## THE CHEMICAL DIAGNOSIS OF PREGNANCY BY DETECTION OF ESTRIN IN URINE\*

M. J. SCHMULOVITZ, A.B., AND H. BOYD WYLLIE, M.D., BALTIMORE, MD.

DURING the course of an investigation of the phenol excretion in pregnancy, we have devised a short method of chemically detecting estrin in the urine of pregnant women. The method consists in the extraction of estrin from the urine with ether and its detection by coupling with diazotized para-nitroaniline (the color reagent) to form a deep colored azo dye, a reaction first noted by Harington and Schüpbach.<sup>1</sup> The term *estrin* is used in this paper to include *theelin* (ketohydroxyoestrin) and *theelol* (trihydroxyoestrin) and is synonymous with *female sex hormone*, *folliculin*, *menformon*, and *progynon*.

A chemical test for estrin in the urine of pregnant mares<sup>2</sup> has appeared while we were working, based on the extraction of estrin and its reaction with concentrated sulphuric acid to give a fluorescent greenish red color. This reaction is a well-known one, and was first used by Kober<sup>3</sup> in devising a method for the chemical assay of pure theelin. The Kober assay has recently been studied and modified by Cohen and Marrian,<sup>5</sup> and a method for the quantitative chemical estimation of theelin and theelol in urine of pregnant women has been devised by these investigators. We have studied the sulphuric acid fluorescence reaction with human urine extracts prepared as described below and found that it gives a color which is far less distinct and less sensitive, and by far less easy to estimate colorimetrically, than that given by the diazo reaction.

\*From the Department of Biological Chemistry, School of Medicine, University of Maryland.

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An extract from a paper presented, by invitation, before the Baltimore City Medical Society, Section of Obstetrics and Gynecology, March 8, 1935.

The substances ether extractable from acidified urine which may react with the color reagent are, essentially, all phenols of different types esters, free volatile phenols (normally almost none), nonvolatile phenols, phenols conjugated with sulphuric and glycuronic acids, and aromatic hydroxy acids. These phenols are shown in analytic classification in Fig 1

#### PLACENTS EMPLOYED

*Ether*—Merck's anesthetic ether (U.S.P. X) is used throughout

*Sodium Carbonate Solution*—Twenty per cent (wt. by vol.) Baker's technical anhydrous in distilled water Sp. Gr. 1.1438 at 23° C

*Saturated Sodium Carbonate Solution*—Baker's technical anhydrous in distilled water Sp. Gr. 1.22 at 23° C

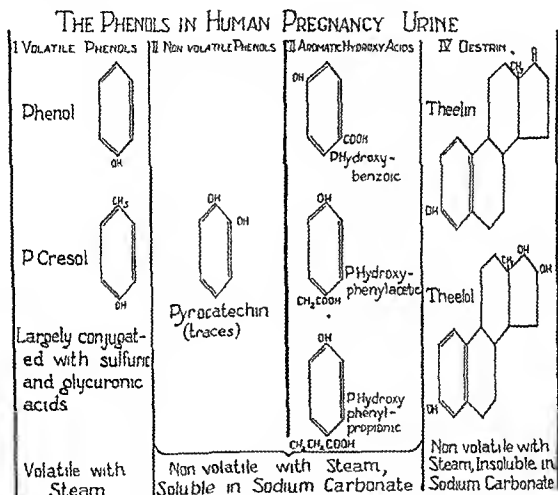


FIG 1

*Stock Sodium Nitrite Solution*—Five per cent Twenty five grams of Baker's analyzed 86.3 per cent  $\text{NaNO}_2$  dissolved in water and diluted to 500 cc

*Stock p Nitroaniline Solution*—Dissolve 15 gm p nitroaniline (Eastman, No. 179) in 500 cc of water containing 40 cc of conc.  $\text{HCl}$

*Ferric Chloride Solution*—Thirty three per cent Five hundred and fifty grams of  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  (Baker's Analyzed) dissolved in water and diluted to 1,000 cc

#### PREPARATION OF P-NITRODIAZONIUM CHLORIDE SOLUTION

##### (The Color Reagent)

Twenty five cubic centimeters of the stock p nitroaniline solution are added to 15 cc of the stock solution of sodium nitrite. This solution is prepared fresh daily. At room temperature or in ice water, this solution slowly becomes cloudy, but in the refrigerator at about 5° C it remains clear indefinitely.



## METHOD AND PRINCIPLES

The twenty-four hour urine specimen is brought to pH 4 with concentrated hydrochloric acid and evaporated down to about 200 c.c. This treatment "deconjugates" the conjugated volatile phenols and volatilizes almost completely the volatile phenols thus formed. This volatilization has been verified by control experiments with pure solutions of phenol and p-cresol.

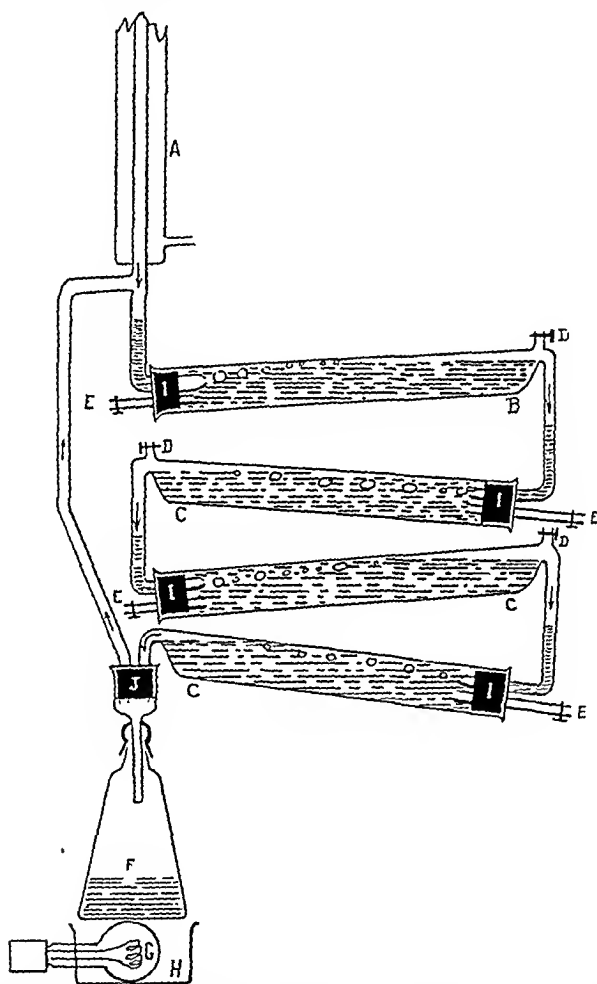


Fig. 2.—A, Condenser; B, Urine extraction tube, 300 c.c. capacity; C, Sodium carbonate washers, 100 c.c. capacity; D, Air vents; E, Inlet-outlet pinch-clamps; F, Ether; G, 60-watt lamp; H, Asbestos-lined can; I, Rubber stoppers; J, Cork stopper.

The acid hydrolysis also releases the hormone from an ether-insoluble ester combination (probably with sulphuric acid) as Marrian<sup>4</sup> and Cohen and Marrian<sup>5</sup> have shown.

The urine is then brought to pH 1 with concentrated hydrochloric acid, since it has been shown<sup>6, 7</sup> that the extraction is more efficient at this concentration, and the hormone, together with the remaining phenols, is then extracted with 150 c.c. ethyl ether. For this purpose we have devised an

apparatus (Fig 2) which not only continuously extracts the urine but also automatically washes the ether extract with sodium carbonate solution, thus removing most of the aromatic hydroxy acids and pyocatechin. We generally allow the extraction to proceed from 5 P.M. one day to 9 A.M. the next.

The ether extract is washed with 10 c.c. portions of 20 per cent sodium carbonate until no further change is seen when 1 c.c. of the color reagent is added to the wash, and then with saturated sodium carbonate until the wash gives a negative test with 1 c.c. of the color reagent. This washing quantitatively removes aromatic hydroxy acids as Tisdall<sup>8</sup> has shown. We have found that it also removes pyocatechin. However, sodium carbonate does not remove estrin from ether for it is used in this way in many isolation procedures.<sup>4</sup> A 10 c.c. wash with 2 per cent hydrochloric acid and a 10 c.c. wash with distilled water conclude the washing of the ether.

Volatile phenols still remaining in the ether extract are now removed by distilling off the ether, adding water to the residue and distilling until the distillate (made alkaline with sodium carbonate) no longer gives a positive reaction with the color reagent. This procedure quantitatively volatilizes phenol and cresols as Hanke and Koessler<sup>9</sup> have shown. The last traces of water are removed by heating in a glycerin bath at 160° C.

The hormone residue is now dissolved in 75 c.c. of 95 per cent ethyl alcohol with warming. This is cooled, 1 c.c. of the color reagent is added, and 15 c.c. of 20 per cent sodium carbonate are added. The flask is well swirled and the contents are transferred to an 8 × 1 inch test tube. The alcohol-carbonate phases spontaneously separate and the presence of estrin is indicated by a dark orange to deep wine colored alcoholic layer over a colorless carbonate layer. When the urine contains very little estrin, as in the urine of males and nonpregnant females, the alcoholic layer is yellow to pale brown with no red tint.

For the purpose of permanent record, the colored alcoholic layer is carefully pipetted off, filtered into a dry colorimeter cup, and placed at 10 mm. This is matched against a 33 per cent ferric chloride solution, the reading of which is recorded as the "ferric chloride number" (FN). In late pregnancies in which the estrin excretion is accordingly higher it is sometimes necessary to place the colored alcoholic test solution at 5 or 25 mm., such readings are, respectively, doubled or quadrupled. For purpose of pregnancy diagnosis, we may say that an FN below 15 is considered negative and above 25, positive. Numbers between 15 and 25 are doubtful and have been obtained in very early pregnancy, in cases of dead fetus, and in 1 case of menopause.

#### RESULTS

Our chief interest in this method has been for the chemical diagnosis of pregnancy, for which purpose we have used it in various forms, for the past year and a half. The results of 89 analyses made on 56 pregnant and non pregnant individuals are summarized in Table I. The detailed clinical results will be discussed more fully elsewhere.

An especially interesting fact is that we have been able to confirm Smith and Smith's<sup>12</sup> finding of a considerably diminished excretion of estrin in the toxemias. Our series includes 5 cases of prepartum and intrapartum eclampsia and the estrin excretion in these cases has been even lower than that of twelve-week normal pregnancies. In cooperation with the Department of Obstetrics of the University Hospital, we are now studying the value of this test as a method of diagnosing impending eclampsia, and the effects of theelin treatment in such cases.

TABLE I

	56 INDIVIDUALS	89 URINALYSES
Known Pregnant	38	58
Positives	37	53 <sup>6</sup>
Doubtfuls	1 <sup>1</sup>	5 <sup>2</sup>
Negatives	0	0
Known Nonpregnant	22 <sup>3</sup>	31
Negatives	16	24 <sup>7</sup>
Doubtfuls	5 <sup>4</sup>	6 <sup>4</sup>
Positives	1 <sup>5</sup>	1 <sup>5</sup>

<sup>1</sup>Two or three weeks pregnant, but not confirmed clinically until two months later. A later specimen not available.

<sup>2</sup>(1) - Same as <sup>1</sup> above.

(2) - Doubtful at four days past the expected menses, positive ten days later.

(3) - Doubtful at ten weeks, bleeding one day monthly, positive at twelve weeks, therapeutically aborted at fourteen weeks.

(4, 5) - Aged forty-two years, doubtful tests at seven and eight and one-half weeks, Mazer-Hoffman test negative; positive at eleven weeks.

<sup>3</sup>Includes four postpartum urines of individuals also studied before delivery.

<sup>4</sup>Six urines of five patients:

(1, 2, 3) - Missed abortions, two before and one after delivery.

(4) - This patient had in the left lower quadrant of the abdomen a twenty-six-week-old fetal skeleton which had been there for three years.

(5) - Menopause, nervous symptoms intense.

(6) - Ten days postpartum.

<sup>5</sup>Six week menstrual cycle, 4,760 c.e. (48 hr. spec. ?), two days before menses.

<sup>6</sup>Distribution of 53 pregnancy urines.

9 - 3 to 4 wk. pregnant

8 - 5 to 8 wk. pregnant

21 - 3 to 6 mo. pregnant

14 - Before delivery

1 - 16 wk. pregnant but menstruating regularly

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53

<sup>7</sup>Distribution of 24 negative urines.

2 - Normal, nonpregnant females

5 - Normal males

4 - Menopause

1 - Missed abortion, before delivery

1 - Missed abortion, after delivery

5 - Normal postpartums

1 - Large ovarian cyst

1 - Uterine fibroid

1 - Cervical polyp

1 - Ectopic pregnancy, two days preoperative

1 - Menses delayed eight days

1 - Endocrinopathy

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24

## DISCUSSION

The sensitivity of the diazo coupling reaction may be recognized when we recall that Hanke and Koessler<sup>9</sup> showed that as little as 0.001 mg of phenol can be estimated quantitatively with it (they used diazotized sulphamic acid). Furthermore, examination of Hanke and Koessler's data will show that the reaction is stoichiometric, that is equimolar quantities of different phenols give the same amount of color production. Hence, theelin and theelol, with a molecular weight 3 times that of phenol, theoretically should give an estimable color production in quantities as small as 0.003 mg. (We have found, in work in progress on a quantitative chemical method of estimating estrin, that 5 rat units of pure theelin gave a quantitatively measurable color production.) Now, when it is recalled that Cohen and Marrian<sup>5</sup> have shown that urine of women late in pregnancy contains 10 mg theelol and 0.1 mg theelin per liter, and that even women in the first six weeks of pregnancy excrete 300 to 600 mouse units of estrin,<sup>10</sup> the depth and sensitivity of this reaction is not surprising.

In addition to the acidic phenols, neutral substances are also ether soluble from acid urine. "The two principal neutral substances which are so far known to occur in human pregnancy urine (are) cholesterol and pregnandiol."<sup>11</sup> Cholesterol was tested and found not to react with the color reagent, pregnandiol was not available for testing but would hardly be expected to react.

Histidine, which has been found in pregnancy urine in increased quantities,<sup>11</sup> has also been subjected to our procedure. As we expected, it was found to be ether insoluble from the hydrochloric acid in which it forms the hydrochloride. This is true of the aromatic and heterocyclic amino acids in general.

Volatile phenols were originally removed from the ether by washing with 25 per cent sodium hydroxide. Although extracts so washed were still decidedly estrogenic, it is quite likely that this wash removed most of the theelol, for Cohen and Marrian<sup>5</sup> have shown that N/10 sodium hydroxide removed theelol from ether almost quantitatively while N/1 sodium hydroxide (about 4 per cent) is required to remove theelin. The first thirty-two urinalyses were done by the sodium hydroxide wash method and the "ferrie chloride numbers" given by the pregnancy urines in that series are considerably lower than those given with the steam distillation method used thereafter, evidence of the fact, we believe, that theelol was removed by 25 per cent sodium hydroxide.

The ether extract is generally colored light yellow by urine pigments, and this color largely persists even after the washing. Treatment of the extract with Norite A (Pfanstiehl) completely removes the pigments, but, judging from the color tests obtained with divided pregnancy extracts in which one portion was so treated, we believe hormone is also lost. We, therefore, do not advocate its use at present.

## SUMMARY

A method is described for the chemical detection of theelin and theelol in urine of pregnant women. Results obtained with this method in 89 urinalyses of 56 patients suggest that it may be used for chemical diagnosis of pregnancy.

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Note added in proof, November 1, 1935: Since this paper was accepted for publication, several small modifications of the procedure have been made. We now use a much simpler extraction-washing apparatus—a description of this will appear in *Science*. The washing of the extract is done with a stirrer in a cylindrical separatory funnel. The final drying is carried out in a water-bath under vacuum rather than in the glycerin bath at 160° C. These changes described in detail together with results on 200 urinalyses to date will be the subject of a subsequent paper.

The authors will be glad to send a description of these changes in procedure to anyone on request.

# DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

**BANTI'S DISEASE**, Relation of Chronic Congestive Splenomegaly to, Larrahee, R. C. Am J M Sc 188 745, 1934

On the basis of a study of 47 cases the view is expressed that in the majority of patients presenting the clinical picture of Banti's disease (splenomegaly with fibrosis, microcytic anemia with leucopenia and a late stage with hemorrhages and ascites) the condition is dependent upon various intraabdominal lesions obstructing the venous outflow of the spleen. By far the commonest of these is liver cirrhosis of various types. As Banti has limited the definition of the disease which bears his name in such a manner as to exclude these cases it is thought best to segregate them under a distinctive name. However, splenectomy is indicated just as in Banti's disease, regardless of the nature of the underlying lesion.

**JAUNDICE** Study of One Hundred Cases of With Particular Reference to Galactose Tolerance, Schiff, L., and Senior, F. A. J A M A 103 1924, 1934

The galactose tolerance test was positive (output of galactose exceeded 3 gm) in forty nine of fifty cases of catarrhal jaundice and fourteen of fifteen cases of acute toxic hepatitis.

A negative test (output of less than 3 gm) was obtained in all of twenty cases of obstructive jaundice. Negative tests were mostly obtained in cases of cirrhosis and neoplasms of the liver.

The test appears of great value in differentiating acute (toxic or infectious) jaundice from obstructive (extrahepatic) jaundice.

The galactose tolerance bears no direct relationship to the degree or duration of jaundice or to the amount of retention of bromsulphalein. In the later stages of acute intrahepatic damage it may remain positive when other tests of liver function have become negative.

The test should be repeated when a discrepancy arises between clinical and laboratory observations.

**SPUTUM**, Study of, in Pulmonary Asbestosis, Page, R. C. Am J M Sc 189 44, 1935  
From a study of 38 cases the author concludes that

1 The presence of asbestos bodies in the sputum is indicative merely of exposure to asbestos dust. If they are of large size it means that a long interval has elapsed since the onset of exposure.

2 The number of bodies in any given specimen is insignificant, but the presence of old and weathered bodies on repeated examinations strongly suggests that a definite pathologic process is in existence.

3 Clumps in the sputum are definite evidence of lung disintegration, but their absence does not mean that disintegration is not in process.

4 Elastic fibers are probably indicative of rapid lung destruction.

5 Elastic fibers may be present in the sputum in pure pulmonary asbestosis both with and without clumps, or in asbestosis with associated tuberculosis.

6 The routine examination of the sputum in cases of suspected pulmonary asbestosis is essential, as it plays a significant role in the clinical diagnosis.

**SEDIMENTATION TEST**, Standardized Technic for, Wintrobe, M. M., and Landsberg, J. W. *Am. J. M. Sc.* 189: 102, 1935.

The following method is recommended as satisfactory and accurate:

1. Five cubic centimeters of venous blood is collected by means of a dry syringe and needle and mixed in a small bottle containing 4 mg. solid potassium oxalate and 6 mg. solid ammonium oxalate. This concentration of oxalate does not alter the sedimentation rate as compared with that of blood collected in heparin. Less than 1 c.c. of blood is needed for the sedimentation test. The remainder can be used for other blood examinations.

2. The blood so collected should be used for the determination of sedimentation rate within four hours of its time of collection. Further delay may be associated with increased suspension stability of the blood.

3. The hematocrit is filled to the 10 cm. mark with blood. The upper level of sedimenting corpuscles may be read at frequent intervals or, more simply, a single reading may be made at the end of 1 hour.

4. Since sedimentation rate increases with increasing temperature, the sedimentation test should be carried out at a temperature not less than 22° nor greater than 27° C. Within this range variations resulting from differences in temperature are small. If the blood used has previously been kept in a refrigerator it should first be permitted to attain the above temperature before being used.

5. The hematocrit should be kept in an exact vertical position during the sedimentation of the blood corpuscles, for when the instrument stands at an angle of even 3° from the vertical, significant acceleration of sedimentation takes place.

6. After sedimentation rate has been determined, the hematocrit containing the blood should be centrifugalized and volume of packed red cells determined. The sedimentation rate may then be corrected for alterations due to anemia. A correction chart is presented.

**PNEUMOCOCCUS**, Sodium Desoxycholate for the Identification of, Lefson, E. J. A. *M. A.* 104: 213, 1935.

Method: 2 drops of a 10 per cent aqueous solution of sodium desoxycholate are added to 1 c.c. of pneumococcus culture. Lysis occurs in two to five minutes. The pH of the culture must not be below 6.5 or the reagent precipitates, and the temperature at which the test is carried out should be below 50° C.

(The reagent can only be obtained in this country from Riechel-deHaen, New York). The reaction is not seen with any strain of streptococci thus far tested.

**PNEUMOCOCCUS AND STREPTOCOCCUS VACCINES**, Cutaneous Reactions of Children to, de Bruin, M., and Vedder, A. *Am. J. Dis. Child.* 48: 791, 1934.

Infants younger than three months react weakly or not at all to intracutaneous injections of vaccines of dead pneumococci and streptococci.

Older infants and young children react in a gradually increasing percentage of cases.

No type-specific or species-specific differences between the vaccines were observed. Vaccines of pneumococci and green-producing and hemolytic streptococci act the same.

The reaction is specific for the group of pneumococci and streptococci.

Children who have suffered from bronchitis, lobular pneumonia, and other conditions in which pneumococci and streptococci are of etiologic importance often give a positive reaction; other children, a negative reaction. Possibly as a result of such a condition a negative reaction changes into a positive one.

Older children and adults (students) in the majority of cases react positively.

Children who have suffered from lobar pneumonia, acute rheumatic fever and chorea almost never react to the vaccines.

In persons having suffered from erysipelas the percentage incidence of negative reactions is the same as the chance of recurrence

In erysipelas a positive reaction points to immunity, a negative one indicates that a chance of recurrence exists. In lobar pneumonia and rheumatic fever also some relation perhaps exists between a negative reaction and the incidence of recrudescence

The skin reaction has a significance analogous to the tests of Pirquet and Mantoux

#### GONOCOCCUS The Criteria of Cure of Gonorrhea in the Male, King, A J J A M A 104 178, 1935

Attention is called to the value of the oxidase test to the presumptive recognition of gonococcus colonies

Flood the surface of the medium with a freshly prepared 1 per cent solution of dimethyl paraphenylenediamine hydrochloride in distilled water

Colonies showing the characteristic color reaction (a pink coloration deepening through purple to jet black in about thirty minutes) are selected for gram staining. The test is not specific, other members in the neisseria group reacting, but only the *M. catarrhalis* closely simulates the reaction given by the gonococcus

False positive reactions are usually given by a thin filiform type of *B. coli* and also by *B. subtilis* but easily detected on microscopic examinations

#### EMPHYEMA Putrid, With Special Reference to Anaerobic Streptococci, Fisher, A M, and Ahernethy, T J Arch Int Med 54 532 1934

Attention is called to the fact that infections due to anaerobic streptococci may occur frequently and fail to be recognized unless cultivation is properly performed

A case is reported of putrid empyema with pulmonary abscesses septicemia and metastatic putrid abscesses due to a gas producing facultative anaerobic streptococcus

Other cases of putrid empyema are reported in which no anaerobic streptococcus was found in conjunction with other organisms

Anaerobic streptococci were found to be the predominant organisms in 2 cases of pulmonary abscess in which cultures were made directly from the pus at operation. Other authors have stressed the frequent occurrence of these organisms in such lesions

Although the finding of anaerobic streptococci in cases of pleural and pulmonary disease has been emphasized, the literature indicates their frequent occurrence in other infections, notably in cases of puerperal sepsis or septic abortion, otitis media, pulmonary abscesses, and hepatic abscesses

Mention is also made of a case of pyelo phlebitis with multiple hepatic abscesses due to an anaerobic streptococcus. An attempt is made to identify the streptococci reported with various types described in the literature. They do not completely fulfill the characteristics of any one group

Cultures of one strain were capable of producing empyema in rabbits when injected intrapleurally. Other strains gave varying results under similar conditions

Evidence is presented to indicate that these streptococci may often be pathogenic for man and for animals

#### BREAST LESIONS, Clinico Pathological Relationship in Common, Lamb, F H Am J Clin Path 4 327, 1934

A knowledge of the clinicopathological relationships in breast disease is the basis for its intelligent management. The simple classification of benign and malignant tumors, cystic and inflammatory disease covers the vast majority of lesions with which one may be confronted. Some of the natural limitations in the differential diagnosis of the most common breast lesion, namely the solitary mobile nodule, have been emphasized in a statistical way. It has been pointed out that multiple lesions in one or both breasts are likely to be benign solid tumors of a type of cystic disease, and the rationale of simple



excision in the former case and mastectomy in the latter has been explained. Personal experience in the immediate examination of breast tumors has demonstrated repeatedly the advantage of this form of cooperation between the surgeon and pathologist. An exploratory operation on the breast should not be undertaken unless one is fully prepared to perform a radical operation if malignant disease is found.

**BRONCHIECTASIS, Morphologic Varieties of, in the Adult, Bendove, R. A., and Gershwin, B. S. Arch. Int. Med. 54: 131, 1934.**

Recent studies of the respiratory dynamics of the bronchi and their defensive or expulsive mechanism are briefly reviewed and the physiopathologic changes which may precede or accompany bronchial dilatation are discussed with especial reference to their diagnostic significance in early bronchiectasis.

Six distinct morphologic types of bronchial dilatation, five of which are bronchiectatic and one of which is bronchiolectasis, are described as visualized roentgenologically after the intrabronchial injection of iodized oil. They are: the tubular, the cylindric, varicose and globular types, bronchiolectasis and bronchiectatic abscess.

The various theories concerning the mode of production of the polymorphic varieties of bronchiectasis are advanced, and the probable pathogenesis of each type is discussed. The tubular form may result from prolonged irritation of the bronchial mucosa; sclerotic and degenerative changes of the myo-elastic layers are considered to be the primary lesion in the cylindric form; the varicose type is, as a rule, brought about by massive pulmonary cirrhosis or atelectasis, whereas a diffuse bronchopulmonary distribution of fibrosis may entail bronchiolectasis; the globular type is only a segmental pouching occasioned by local weakening in the bronchial wall and the primary bronchiectatic abscess is initiated by suppurative lesions in the bronchi.

Each of these morphologic types reveals itself clinically by a different mode of onset, a variable train of symptoms and certain physical signs. The clinical history is of greater diagnostic value than are the physical signs. Further studies and closer correlation between symptoms, signs and morphologic aspects as outlined by iodized oil in the living will no doubt enable us to draw a sharper line of clinical demarcation between the various forms.

No diagnosis of bronchiectasis is complete unless the location, the distribution and, above all, the type have been determined, for on this information often depends the therapeutic procedure to be followed.

**BLOOD: Relationship of the Intrinsic Factor to a Hematopoietic Material in Concentrated Human Gastric Juice, Helmer, O. M., Fouts, P. J., and Zervas, L. G. Am. J. M. Sc. 188: 184, 1934.**

This is a continuation of previously reported studies upon the increased potency of liver extract No. 343 after incubation with 100 c.c. of normal gastric juice. (Minof, G. R., and Murphy, W. P., J. A. M. A. 87: 470, 1934.)

The purpose of the present investigation was to study the intrinsic factor responsible and to attempt to determine some of its characteristics. The following conclusions are advanced:

The active principle necessary for the maturation of red cells is present in the liver at least 2 months before birth.

The active principle may be absent from the liver of an inadequately treated case of pernicious anemia.

The active principle is present in the liver of an adequately treated case of pernicious anemia.

A cirrhotic liver may not contain the active principle.

It is highly suggestive that a liver may be sufficiently damaged so that the active principle, though present, cannot be presented to the tissues for utilization.

A pernicious anemia like blood picture may be present in a patient, if the liver is so damaged that it cannot store the active principle, or cannot present it to the body tissues in the proper form for utilization

The demonstration that all livers do not contain the "active principle" indicates that it is a storage product rather than an intrinsic part of the liver substance

**RHEUMATOID ARTHRITIS, Treatment of Chronic, With Streptococcus Vaccine, Wainwright, C W J A M A 103 1357, 1934**

Ninety four blood cultures in 51 cases of rheumatoid arthritis yielded *Streptococcus viridans* in 1 case, diphtheroids in 4 cases staphylococci in 4 cases and gram positive bacilli in 3 cases

Fourteen joint cultures in 14 cases of rheumatoid arthritis were negative

The serums of 46, or 90 per cent of 51 cases of rheumatoid arthritis were found to possess agglutinins for hemolytic streptococci

All of 55 cases of rheumatoid arthritis gave positive skin reactions to one or more strains of streptococci

Twenty one of 28 cases of rheumatoid arthritis have shown improvement following intravenous injections of streptococcus vaccine prepared from the strain to which the skin was most sensitive

**CANCER, Roffo's Test in Statistical Results of 1,100 Cases, Gandolfo, A Am J Cancer 22 363, 1934**

Though Roffo's test is not specific, it is of value as an auxiliary method in the diagnosis of cancer

On account of the simplicity of its technic it can and should be currently used in clinics

Though a negative Roffo's test does not exclude the presence of a tumor, a positive result should induce us to continue investigations, in order to discover the tumor, as the proportion of erroneous positive results in this test is small (6.37 per cent in 6,718 tests undertaken)

Roffo's test has given a high percentage of positive results in cancer of the uterus (68.33 per cent), ovary (78.94 per cent), bladder (72.41 per cent), stomach (76.26 per cent), intestine (76 per cent), liver (84.20 per cent), pancreas (84.61 per cent), lungs (75 per cent), and mediastinum (86.66 per cent) and in osteosarcomas (71.42 per cent), all of which generally offer greater difficulties in clinical diagnosis

**ALBUMINURIA Functional, Analysis of 58 Cases With Results of Thyroid and Calcium Medication, Burden, N J Am J M Sc 188 242, 1934**

Nothing was found in the past medical histories or in the physical examinations of 56 otherwise healthy male students with persistent functional albuminuria to account for the urinary abnormality. The kidney function as determined by the phenolsulphone phthalein and Mosenthal tests was normal

Cardiorenal disease in the parents of students with functional albuminuria was more than twice as frequent as in the parents of students showing no such abnormality

A tendency to low basal metabolism readings was found in 80.9 per cent but in the 10 cases tested with moderate doses of thyroid extract there was no improvement in the albuminuria

The blood serum calciums were normal in 10 students with persistent functional albuminuria, although calcium medication had a favorable influence on the albuminuria in 11 of 16 cases tried

As heretofore, Jordan's *Bacteriology* can be recommended as comprehensive, well planned, equally well executed, and authoritative.

It will without doubt retain the place it has won as a practical and useful standard reference text.

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### Textbook of Bacteriology\*

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\*Textbook of Bacteriology. By Thurman B. Rice, M.D., Professor of Bacteriology and Public Health at the Indiana University School of Medicine. Cloth, pp. 551, Figs. 121. W. B. Saunders Co., Philadelphia, Pa.

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## REVIEWS

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Books and Monographs for Review should be sent direct to the Editor,  
Dr Warren T. Vaughan, Professional Building, Richmond, Va

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### Disorders of the Blood<sup>\*</sup>

DESPITE the importance of the blood as a specialized tissue, primary disease of the blood per se is relatively uncommon. On the other hand, the varied functions of the blood as the great common carrier of the body render inevitable its association with disease in general so that changes in the peripheral blood constitute important evidence of disease or disorder of other systems of the body.

The title of this book, *Disorders of the Blood* has been chosen with this thought in mind and indicates the comprehensive nature of its contents.

The volume begins with a discussion of the origin, developments, and functions of the blood cells and also of the abnormal cells found in the circulation. This is followed by the principles and practice of hematologic diagnosis. This is followed by discussions first of diseases arising primarily in the blood itself and the hematopoietic system, and then by discussions of the various changes associated with other diseases as a reflection or indication of which disturbances occur in the blood.

The authors have attempted to reduce the hematologic literature of the past decade to a reasonable compass but, when necessary, have not hesitated to comment on or expand it from the aspect of their own experience which, obviously, has been extensive.

At the end of each chapter the contents are summarized briefly and to the point.

The style is clear and without verbosity or ambiguity. The illustrations are well chosen and reproduced.

An excellent index and a separate index of authors complete a well planned and well-executed volume which may be recommended heartily as an authoritative and comprehensive reference text upon disorders of the blood.

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### Clinical Diagnosis by Laboratory Methods†

TODD and Sanford's *Clinical Diagnosis by Laboratory Methods*, used for years, certainly needs no introduction.

The eighth edition is outstanding with its several advances. First, rearrangement of the chapters and bringing the subject matter together more compactly and intelligently is a vast improvement. Second, the book has necessarily been enlarged to include entirely new methods and improved illustrations. Of particular interest is the new section on hemoglobin, including the Wintrobe, Haden Haussner and Osgood Haskins hemometers. The new chapter on clinical chemistry, including determination of sulphhemoglobin, the Exton and Rose method for sugar tolerance, a simple test for carotinaemia, cholesterol, lecithin and total lipid in blood plasma will be found very useful.

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<sup>\*</sup>*Disorders of the Blood*. By James Campbell Todd, Ph.D., M.D., Late Professor of Clinical Pathology, University of Colorado, School of Medicine and Arthur Hawley Sanford, M.D., Professor of Clinical Pathology, University of Minnesota (The Mayo Foundation). Head of Section on Clinical Laboratories, Mayo Clinic. Eighth edition thoroughly revised with 370 illustrations, 29 in colors, pages 792. W. B. Saunders Company, Philadelphia, 1935.

<sup>†</sup>*Clinical Diagnosis by Laboratory Methods. A Working Manual of Clinical Pathology*. By James Campbell Todd, Ph.D., M.D., Late Professor of Clinical Pathology, University of Colorado, School of Medicine and Arthur Hawley Sanford, M.D., Professor of Clinical Pathology, University of Minnesota (The Mayo Foundation). Head of Section on Clinical Laboratories, Mayo Clinic. Eighth edition thoroughly revised with 370 illustrations, 29 in colors, pages 792. W. B. Saunders Company, Philadelphia, 1935.

In addition there are such valuable addenda as Van Allen's thrombocytoerit, the Nygaard plasma platelet count, the Wintrobe and Lunsberg sedimentation method, the rose bengal test, a quick method for preparation of colloidal gold as well as new methods in serology

There are twenty five new illustrations, well placed

### Clinical Atlas of Blood Diseases\*

THOSE familiar with this very useful atlas will welcome its third edition. Those who are not will find it of great and valuable assistance in the study of diseases associated with disturbances of the blood.

As advances in the field of hematology have been greater in the clinical than in the morphologic field, the plates of this edition are the same. The text, however, has been revised and largely rewritten. It may be questioned whether the exceedingly brief section on hematologic methods which has been added increases the value of the book appreciably because of its brevity. With this minor criticism, the volume remains as before, a practical, useful and valuable contribution to the study of the blood.

### Doctors and Juries†

IN THIS small book, the author has compressed more sound advice based on common sense and practical experience than appears on the surface, in many larger volumes covering similar ground.

As expressed in the preface "This book is for doctors who desire to know something of the legal aspects of their profession, and for lawyers and others who wish information concerning the relationships of the law with the practice of medicine."

It is to be hoped that among these it will have a wide circulation for it well repays reading and rereading by all who appear in court—not only the doctor who makes practically a business of such appearances but especially by those who may, and often do, find themselves in court unexpectedly or even unwillingly.

While the author, as a lawyer, may not, perhaps, subscribe without reservation to the famous dictum laid down by Mr Bumble, to wit "The law is an ass!" he has not hesitated to comment upon the absurdities consequent upon legal technicalities.

To this reviewer, at least, it is refreshing to read a book on this subject which does not appear to be based upon the premises that all courts are majestic in their operation, all judges learned, and all juries intelligent, not to mention the assumption that all doctors are necessarily good or useful witnesses.

If all physicians appearing or about to appear in court were thoroughly familiar with the chapters on Testifying, Expert Witnesses, Hypothetical Questions, and Influencing Juries the spectacle would be much improved in dignity and effectiveness. And every physician if he does no more, should read the chapter on Observations which, appropriately enough, begins with "keeping out of trouble."

Thus is a valuable book, not only for the doctor but for the lawyer as well.

### Textbook of General Bacteriology‡

THIS volume needs no introduction. That it has now reached an eleventh edition is ipso facto evidence of its usefulness and worth.

The present edition has been extensively revised, largely rewritten, and entirely reset and the book now reflects the many important changes and additions which have occurred in this field since the last edition of four years ago.

\*Clinical Atlas of Blood Diseases. By A. Piney, M.D. Assistant Physician St. Mary's Hospital for Women and Children, London, and Stanley Ward, M.D. Physician The Cancer Hospital, London. Cloth pp 110 ed 3 34 plates in color 4 figures. P. Blakiston's Son & Co. Inc. Philadelphia, Pa.

†Doctors and Juries. By Humphreys Springstrun. Cloth pp 135. P. Blakiston's Son & Co. Philadelphia, Pa.

‡Textbook of General Bacteriology. Edwin O. Jordan, M.D. Professor of Bacteriology, University of Chicago and Rush Medical College. Cloth ed 11 pp 825. Figs 202. W. B. Saunders Co. Philadelphia, Pa.

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## CLINICAL AND EXPERIMENTAL

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### THE BEHAVIOR OF THE EOSINOPHILES IN RHEUMATIC FEVER\*

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GEORGE FRIEDMAN M D AND EDWARD HOLTZ M D, NEW YORK, N Y

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**A**LTHOUGH the source and function of the eosinophilic polymorphonuclear leucocytes are not definitely known, certain characteristics of their behavior in the peripheral circulation during the course of infections have been described. It has been clearly established that quantitative diminution of the eosinophiles is found during the height of many febrile diseases, such as pneumonia, typhoid fever, erysipelas, acute appendicitis, etc.<sup>1, 7</sup> Subsequently, in these infections, their number increases, frequently reaching a level higher than normal. In scarlet fever the hypoeosinophilia is of very short duration and hypereosinophilia appears considerably before defervescence. In acute infections, other than scarlet fever, the eosinophilic rhythm is (1) disappearance—fastigium, (2) reappearance—defervescence, (3) transient eosinophilia—convalescence. The presence of eosinophiles and eosinophilia during the course of acute febrile diseases are universally accepted as favorable prognostic signs.<sup>1, 3, 4, 8, 11</sup>

In chronic infections the eosinophiles do not behave with the same regularity. For example it has been shown by Bezaneon de Jong, and de Sorbonnes<sup>12</sup> that in tuberculosis, the often fatal acute pneumonic forms are accompanied by persistent aneosinophilia or hypoeosinophilia, but in chronic tuberculosis with the usual exacerbations, the eosinophilic rhythm is similar to that in acute fevers with favorable prognosis, i.e. the hypoeosinophilia is succeeded by eosinophilia. There is, however, one important difference, viz.,

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\*From the Medical Service and Out Patient Department of the Sydenham Hospital.  
Received for publication February 2, 1935.

†Aneoinophilia refers to the absence of eosinophiles in a routine count of 100 cells.



in nonspecific acute infections, the eosinophilia is comparatively short in duration, but in tuberculosis it frequently persists for weeks and sometimes even for months. It is seen not only at the end of acute episodes but also in the intervals between them.

Eosinophilia has already been described in rheumatic polyarthritis.<sup>6-9, 13</sup> Tuerck<sup>8</sup> and Schwarz<sup>6</sup> observed rising eosinophile counts as the inflammation in any one joint receded, and Tuerck also described convalescent eosinophilia as high as 13.8 per cent. Mayer<sup>9</sup> reported a case with constant eosinophilia in which the course was short and mild and the recovery was rapid. Wile<sup>4</sup> described an early eosinophilia in acute articular rheumatism and also in chorea. Page, Turner, and Wilson<sup>13</sup> found eosinophilia in rheumatic carditis as well as in polyarthritis and chorea. Perry<sup>14</sup> attached little significance to what he termed the usual slight postinfectious eosinophilia with lymphocytosis of acute rheumatic fever and carditis.

The eosinophilic response to rheumatic infection must be further considered in relation to the allergic hypothesis. Swift<sup>15-17</sup> has summarized the evidence pointing to the allergic nature of many rheumatic manifestations, but found eosinophilia in only three of the thirteen cases studied, all of whom had chorea. The relation of eosinophilia to the allergic state in general has long been suspected but its presence is not constant, and it has been impossible to attach specific import to it. In asthma, the eosinophiles are not invariably increased, but their number is more frequently high than in other allergic disturbances.<sup>18</sup> It has even been considered presumptive evidence of allergic etiology in the absence of positive skin tests.<sup>19</sup> Local eosinophilia accompanied by peripheral circulation eosinophilia is known to appear at the site of foreign protein injections.<sup>20, 21</sup> The nasal mucosa is also a site of eosinophile infiltration in asthma and hay fever.<sup>22</sup> Ringoen<sup>23</sup> produced eosinophilia in the peritoneal cavity and blood stream of sensitized animals in which the shock dose had produced an anaphylactic reaction. The linkage of hypersensitivity to eosinophilia is well illustrated in the case of scarlet fever. Not only is eosinophilia present in the early stages of this disease, but it has also been shown by Vaughan<sup>24</sup> that persons with positive Dick skin tests reacted in every case with a rise in eosinophiles to immunizing doses of the specific toxin. In two cases, both with strongly positive skin tests, the eosinophilia was coincident with the appearance of a rash. The same phenomenon has been noted by Kleinberger<sup>7</sup> in response to therapy with tuberculin. In contradistinction to scarlet fever and tuberculosis, where positive skin tests are valuable in indicating sensitivity to a specific organism, in rheumatic fever the value of positive skin tests is considerably diminished because of their inconstant and unpredictable appearance. In rheumatic fever an added difficulty presents itself, viz., no specific etiologic agent has been conclusively demonstrated. Therefore, although we may accept positive Dick tests, tuberculin sensitivity and eosinophilia, when they occur, as direct evidence of bacterial allergy in scarlet fever and tuberculosis, the corresponding phenomena occurring under similar conditions in rheumatic fever can only be accepted as additional indications of the probable presence and importance of hypersensitivity.

The short case histories presented below illustrate four features of the behavior of the eosinophiles during the course of rheumatic infection. These are (1) the disappearance of eosinophiles from the peripheral circulation during accessions of acute polyarthritis and carditis, (2) the reappearance of eosinophiles and eosinophilia during the early stages of recovery, (3) persistent eosinophilia in cases of continued activity, (4) transient hypo or aneosinophilia occurring during minor exacerbations in cases of chronic rheumatic heart disease.

CASE 1—Male, negro, aged forty eight years was admitted to the clinic in March, 1932, complaining of dyspnea, substernal pain, pain in the left shoulder, and cough. These symptoms began two weeks after an acute upper respiratory infection. He had had an attack of arthritis at the age of forty years in which the only joint affected was the left hip. One year later, he had migrating polyarthritis. For the next three years, there were minor joint pains and then a symptom free interval succeeded finally by cardiac decompensation, which, interpreted in the light of later events probably was due to rheumatic carditis. The anatomic lesion was rheumatic mitral and aortic endocarditis. Auriculoventricular conduction was delayed (0.24 sec). He improved slowly and in December, 1932, he was able to resume part time work. During the remainder of the period of observation rheumatic activity was considered to be subsiding but there were transient, mild exacerbations. The prolonged *av* conduction time remained unchanged.

Table I illustrates the behavior of the eosinophiles in the peripheral circulation. The period of slow clinical improvement was marked by normal and elevated eosinophilo counts. The single instance of aneosinophilia was found during a period when signs of increased cardiac embarrassment had temporarily reappeared. This case illustrates the disappearance of the eosinophiles in the routine blood count (100 cells) during exacerbations of acute rheumatic carditis and their reappearance during stages of recovery. It also illustrates the increase of the eosinophiles to a level considerably above normal (8 per cent) during the stage of slowly subsiding active infection, and aneosinophilia during an intercurrent minor exacerbation.

TABLE I

TOTAL LEUCOCYTE AND DIFFERENTIAL BLOOD COUNTS (100 CELLS) CASE 1

DATE	WBC	SEGMENTED BAND	LYMPH	MONO	EOS	BASO
9/23/32	11,500	71	1	27	0	0
10/ 7/32	7,300	70	0	26	3	0
12/16/32	5,700	50	0	40	6	2
1/13/33	8,000	54	10	26	6	2
1/27/33	6,300	52	0	38	4	0
2/24/33	7,000	64	2	26	2	0
5/19/33	7,300	54	2	34	8	2
6/ 7/33	6,400	58	0	34	6	0
7/14/33	7,100	46	7	36	9	0
8/ 9/33	7,100	64	12	18	6	0
9/25/33	6,300	56	8	32	0	0
10/ 6/33	5,950	49	6	38	5	0
11/ 3/33	6,300	44	4	38	6	0

CASE 2—White male, thirty one years old, a blacksmith, was admitted to the hospital May 4, 1934, with acute polyarthritis which involved successively the right knee, ankles, and the wrists. He had fever, leucocytosis, a high percentage of immature polymorphonuclear leucocytes, and increased sedimentation rate.

Defervescence with disappearance of the joint symptoms began a week after admission. The gradual recovery from this infection is evidenced in Table II by the fall in temperature and pulse rate, and best by the gradual decline of the sedimentation rate. The latter shows a gradual decrease from 29 mm to 4 mm in six weeks (Cutler method, one half hour).

These signs were accompanied by gradual clinical improvement and at first by the transient reappearance in the peripheral blood of eosinophiles in small numbers. During the period of established convalescence eosinophiles were constantly present, rising to a slightly increased level of 5 per cent, with the exception of one observation when they were absent. The latter count was made when a rise in temperature, pulse rate, leucocyte count, and immature forms indicated a recrudescence of infection. There was a complete return to normal two days later, all indices again declining, and the eosinophiles reappeared.

TABLE II  
TOTAL LEUCOCYTE AND DIFFERENTIAL BLOOD COUNTS (100 CELLS). CASE 2

DATE	W.B.C.	SEGMENTED	BAND	LYMPH	MONO	EOS.	BASO	COMMENT TEMP PULSE	SED TIME* MM.
5/ 4/34	10,200	58	10	24	6	2		102.0 103.4	96 102
5/ 9/34	15,600	66	19	9	6	0		101.8-102.6	88 96
5/10/34	13,450	62	16	18	4	0		101.0 102.8	84- 96
5/10/34	12,800	60	14	24	1	0	1	100.0 102.0	72- 86
	12,650	52	15	26	4	2	1		
	11,900	52	19	20	8	1			
5/12/34	12,700	49	18	26	7	0		100.8 101.6	70 84
5/14/34	13,100	43	16	31	10	0		99.8 101.0	68 90
5/17/34	12,800	44	18	32	6	0		99.0 99.6	68 76
5/21/34	13,000	61	11	20	6	2		98.4 100.0	70 88
5/25/34	7,200	59	4	31	6	0		99.0 99.6	80 86
5/28/34	8,450	46	2	40	8	4		98.4 99.4	80 84
5/31/34	6,800	46	2	45	3	4		98.6 99.6	80 84
6/ 4/34	7,100	56	2	38	2	2		98.8- 99.0	74- 80
6/ 7/34	6,900	43	6	47	3	1		98.6 99.4	82 90
6/11/34	9,700	54	4	33	4	5		98.8- 99.6	82 84
6/14/34	11,100	62	8	24	6	0		98.6 100.6	80 108
6/16/34	7,200	57	4	30	8	1		98.8 99.4	78- 84
6/18/34	8,000	51	2	34	11	2		98.6- 99.4	76 84

\*Cutler method, one-half-hour rate

This case illustrates the reciprocal relation between the indices of infection and the disappearance and reappearance of eosinophiles in a case of acute rheumatic fever with pre dominant polyarthritides.

CASE 3—Female, aged thirteen years. This patient came under observation with residual joint pains and a mitral systolic murmur following an attack of acute polyarthritides which had involved many joints successively one month before admission. During the one month which she spent in the hospital she became afebrile and the leucocyte count fell from 14,700 to 9,800 (Table III). The presence of eosinophiles and occasional slight eosinophilia accompanied a steady clinical improvement and fall in the total white cell count. An eosinophilia, occurring once, was accompanied by a slight leucocytosis. At discharge, there were

TABLE III  
TOTAL LEUCOCYTE AND DIFFERENTIAL BLOOD COUNTS (100 CELLS) CASE 3

DATE	W.B.C.	SEGMENTED	BAND	LYMPH	MONO	EOS.
3/19/34	14,700	61	4	31	1	1
3/31/34	13,200	46	4	41	4	5
4/ 2/34	11,100	49	3	43	2	3
4/ 6/34	10,500	60	6	30	2	2
4/10/34	10,000	46	4	42	2	6
4/12/34	11,700	54	4	36	4	2
4/14/34	10,700	44	5	49	2	0
4/18/34	9,800	41	9	44	3	3
6/20/34	10,200	43	6	47	4	0
7/ 5/34	11,400	46	4	47	3	0
10/10/34	9,700	62	1	28	5	2

3 per cent eosinophiles. The aneosinophilia in the two subsequent follow up examinations was coincident with an increase in the leucocyte count to 11400.

CASE 4—Female, admitted at the age of eight years for an eczematoid skin eruption, was discovered to have mitral endocarditis during a routine physical examination. There had been transient muscular pains in the legs for the two preceding years. Three years later she again came to the clinic because of pain in the legs. From that time to the present, she has shown continuous active rheumatic infection with dyspnea, palpitation, tachycardia, fever, and hemorrhagic spots in the mucous membranes of the mouth and of the skin. In the more recent observations, there has been some symptomatic improvement, and for the first time in two years, the cardiac rate has fallen below 100 per minute.

This case illustrates a recurrent eosinophilia during the course of continuous rheumatic activity of moderate severity. At no time was aneosinophilia or hypoeosinophilia obtained in eleven observations over a period of thirteen months. Although tachycardia was always present and fever at four examinations the patient's subjective condition was excellent. She always insisted that she felt "fine" even when her pulse rate was 144. The percentage of polymorphonuclear leucocytes was never higher than 62 per cent and immature forms were only increased slightly. It should also be noted that slight monocytosis was present on three occasions. A peak of 14 per cent eosinophiles was found at the last examination, at which time her clinical condition was strikingly good.

The continued presence of eosinophiles with frequent eosinophilia of unusual degree, characterized a case of rheumatic endocarditis in which activity was continuous but not disabling. The absence of leucocytosis and the mild monocytosis confirm the impression of a mild chronic infection and a comparatively good immediate prognosis. This patient may be thought of as being in the recovery stage for thirteen months.

TABLE IV

TOTAL LEUCOCYTE AND DIFFERENTIAL BLOOD COUNTS (100 CELLS) CASE 4

DATE	WBC	SEGMENTED	BAND	LYMPH	MONO	EOS	BASO	TEMP	PULSE
10/ 7/32	10,000	55	6	27	8	4		101.0	116
11/ 4/32	6,200	39	6	49	4	2		100.6	108
12/ 2/32	9,800	48	9	27	6	9	1	99.0	112
1/13/33	8,600	46	5	33	11	8		100.2	144
2/10/33	9,000	42	10	40	4	4		100.6	110
3/10/33	8,400	53		28	10	4		99.0	124
4/ 7/33	9,650	41		56	10	8		99.6	100
5/18/33	9,700	56	6	30	6	2		98.6	116
7/28/33	11,100	39	4	42	6	9		99.4	100
10/20/33	9,950	51	9	28	8	3	1	99.4	120
11/17/33	9,050	60	1	20	4	14	1	99.0	92

CASE 5—A white female fifty-two years of age admitted to the hospital with acute polyarthritis of one month's duration and mitral stenosis and insufficiency. She had had polyarthritis at five years and thirty-five years. The symptoms were dyspnea, palpitation and slight dependent edema for two weeks prior to admission. Auricular fibrillation was present on the first day, after which it was replaced by normal sinus rhythm. Transient fibrillation appeared once more during hospitalization. Fever and tachycardia were present throughout, with remissions and exacerbations and a pleuropericardial rub appeared for a few days. She also had transient partial A-V block.

The record of the blood findings (Table V) illustrates a complete disappearance of the eosinophiles from the blood during the height of the polyarthritis attack. Reappearance of eosinophiles accompanied her first temporary improvement when joint involvement had entirely disappeared. The eosinophiles again disappeared and remained absent during the exacerbated activity of the rheumatic infection of the heart. The last three blood counts showed normal eosinophilic counts together with a marked drop in the polymorphonuclear cells and a rise in the monocytes.

CASE 6—Male, white, thirty-two years of age, admitted to the clinic Jan. 13, 1931 complaining of shortness of breath, pain in the pericardium and left knee joint, and the arms

TABLE V

TOTAL LEUCOCYTE AND DIFFERENTIAL BLOOD COUNTS (100 CELLS). CASE 5

DATE	W.B.C.	SEG- MENTED	BAND	LYMPH.	MONO.	FOS.	TEMP.	PULSE	SFD. TIME MM.
5/ 9/34	8,600	70	8	18	4	0	99.8-101.4	92-100	14
5/14/34	8,600	58	10	20	12	0	100.0-101.4	88- 94	25
5/15/34	10,200	53	13	26	8	0	99.6-101.8	90-100	
5/18/34	6,900	61	16	19	4	0	99.0-100.4	81- 90	12
5/21/34	8,950	67	14	15	3	1	98.4-100.6	84- 92	6
5/28/34	7,400	66	10	18	6	0	99.8-101.0	88- 98	7
5/31/34	7,100	70	10	12	6	2	99.6	86- 88	10
6/ 4/34	8,300	68	10	12	6	4	99.4-102.0	84- 88	18
6/ 7/34	6,600	70	11	12	6	1	99.4-100.0	84- 88	20
6/11/34	5,900	61	12	16	9	0	99.8-100.6	84- 88	15
6/15/34	8,500	62	8	20	10	0	99.4-100.8	82-100	11
6/18/34	11,100	65	16	14	5	0	100.2-100.6	82- 84	19
6/21/34	5,400	64	12	20	4	0	98.6- 99.6	68- 84	25
6/25/34	4,900	54	10	24	10	2	98.8- 99.2	84- 88	10
6/28/34	5,650	46	11	31	10	2	98.8- 99.0	78- 80	13
6/30/34	5,950	40	12	36	10	2	98.8- 99.2	84- 96	18

and soles of the feet. There was no history of antecedent rheumatic infection. Within two years before admission, he had been in hospitals twice for partial decompensations. The anatomic diagnosis was mitral endocarditis.

The blood examinations from January, 1931, to April, 1932, revealed frequent leucocytosis, a relatively high polymorphonuclear count and a high percentage of immature forms. Of eight counts made during this period, eosinophiles were absent five times. After May, 1932, eosinophiles were found in every film examined, reaching 6 per cent on four occasions. Concomitantly, the polymorphonuclear and immature forms maintained a decidedly lower level than during the preceding years. It is considered that the changes in the blood count illustrate a case of very mild rheumatic activity gradually decreasing and reaching a minimal level.

TABLE VI

TOTAL LEUCOCYTE AND DIFFERENTIAL BLOOD COUNTS (100 CELLS). CASE 6

DATE	W.B.C.	SEGMENTED	BAND	LYMPH.	MONO.	EOS.	BASO.
1/15/31	11,300	63	2	22	3	3	0
1/21/31	12,300	62	8	23	3	0	4
1/21/31	8,900	64	9	21	0	3	3
1/22/31	12,400	63	7	24	7	0	2
1/29/31	11,800	60	8	29	2	0	1
11/10/31	8,200	42	10	42	4	2	0
4/14/32	8,200	62	2	30	6	0	0
5/30/32	12,100	52	10	36	2	0	0
9/23/32	8,500	48	3	42	6	1	0
10/21/32	11,600	51	8	32	8	1	0
11/18/32	13,600	52	4	34	6	4	0
1/ 4/33	9,000	62	2	18	10	6	2
2/17/33	11,000	52	4	36	6	2	0
3/24/33	9,000	50	8	28	8	6	0
6/ 2/33	10,250	46	4	38	8	2	2
8/ 1/33	9,350	52	4	38	2	4	0
8/15/33	8,300	53	6	36	4	1	0
11/17/33	12,650	61	5	20	4	6	1
12/29/33	10,600	48	2	41	3	6	0

CASE 7.—Male, admitted to the hospital in December, 1928, at the age of fifteen years for his second attack of polyarthritis within ten months. Mitral and aortic endocarditis were present. At the end of six weeks the acute articular symptoms and the fever had subsided,

and he was discharged to the clinic. Slight exacerbations of rheumatic activity appeared in February and again in April, 1929. In each of these there was fever but no joint pain. In the summer of 1929, he improved but not until late that year was he allowed to resume his school work. Following this there were short bouts of infection in December, 1929, and in October, 1930. In the interim between these two attacks there were no symptoms. In January, 1933, he had another short bout of fever without symptoms, other than slight malaise. He returned to work and since then has had no signs of renewed infection.

TABLE VII

TOTAL LEUCOCYTE AND DIFFERENTIAL BLOOD COUNTS (100 CELLS) CASE 7

DATE	WBC	SMALLER BAND	LYMPH	MONO	EOS	BASO
9/26/29	13,100					
10/10/29	9,600	50	5	35	7	3
10/15/29	9,200	52	4	40	2	2
10/29/29	10,400	48	0	38	10	3
10/30/29	6,400	50	2	42	3	3
6/20/30	9,400	55	2	35	3	2
10/9/30	11,150	40	6	52	2	0
10/21/30	9,400					
10/25/30	9,800					
11/9/31	12,500	46	6	42	2	4
11/17/31	9,200	50	0	44	2	4
1/21/32	8,900					
4/25/32	6,850					
12/3/32	8,800	46	2	36	12	4
5/19/33	12,700	52	4	34	8	2
6/30/33	9,000	43	6	20	6	4
9/8/33	8,700	44	3	39	2	2
10/6/33	8,000	41	4	42	1	2
1/26/34	8,000	41	4	38	10	7

This case is marked by the almost continuous presence of the eosinophiles in the blood. One observation of aneosinophilia was made. The patient was always relatively in good condition throughout the period of observation. Eosinophilia of very mild degree occurred several times reaching a level of 7 per cent at the last examination.

## DISCUSSION

The behavior of the eosinophilic polymorphonuclear leucocytes in rheumatic fever is similar in all respects to that seen in other infections. In acute rheumatic polyarthritis and carditis during the fastigium, there is hypo- or aneosinophilia. This is succeeded by a return of the eosinophiles to the blood with frequent convalescent eosinophilia. The greatest variety of eosinophilic response is seen in patients who have well developed rheumatic heart disease. In these subjects there are found hypoeosinophilia, aneosinophilia and persistent or recurrent eosinophilia. From a study of the above tabulated differential counts however it is apparent that in so called chronic rheumatic heart disease, the presence and absence of eosinophiles and the occurrence of eosinophilia have the same significance as in other infections. The concept of rheumatic heart disease as a chronic infection with frequent acute exacerbations of variable degree offers the key to an understanding of the apparent complexity of the eosinophilic behavior. Thus repeated and continuous absence of eosinophiles from the blood over a period of time was always found to be associated with other evidences of a severe and active infection. In the majority of cases showing continuous aneosinophilia or hypoeosinophilia of

this type, the patient was ill enough to be confined to bed. Conversely, when the eosinophiles were continuously present in normal or increased numbers, the infection was always subsiding. It may be stated that eosinophilia always indicates convalescence, which in rheumatic fever is admittedly very often a protracted process. Finally, occasional aneosinophilia occurring in patients who usually present normal or high counts, undoubtedly indicates miniature exacerbations of activity which give rise to little or no clinical disturbance.

Swift has shown the close resemblance of rheumatic fever to tuberculosis and has pointed out many similarities in the natural history of the two diseases. The close correspondence of the eosinophile behavior in rheumatic fever with that described by the French observers in tuberculosis,<sup>12</sup> affords another point of identity and the analogy constitutes further evidence in favor of the allergic hypothesis.

Continued observation of the behavior of the eosinophiles, over long periods of time in the less acute cases, is a reliable index of activity of rheumatic infection and is valuable as a basis, in some cases, for immediate prognosis and clinical management.

#### CONCLUSIONS

A study of the eosinophiles in acute rheumatic polyarthritis and acute and chronic rheumatic heart disease leads to the following conclusions:

1. In acute rheumatic polyarthritis and carditis, the eosinophiles are absent or diminished in number during the height of the disease.
2. During the stage of recovery, the eosinophiles reappear and there is postinfectious eosinophilia as in other acute infections.
3. Continuous aneosinophilia and hypoeosinophilia over long periods of time occurring in all three types of the disease and their combinations, indicate intense activity of infection.
4. Recurrent and continuous eosinophilia indicate convalescence.

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## THE INFLUENCE OF SUCROSE INGESTION ON AMINO ACID NITROGEN AND UREA NITROGEN CONCENTRATION OF THE BLOOD\*

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WHILE the metabolism of glucose has been subjected to intensive study less attention has been given to that of sucrose. An investigation concerning the influence of alimentary dextrose upon the amino acid nitrogen and urea nitrogen content of the blood has been reported in a recent paper in this journal.

By the use of similar experimental methods 26 patients have been subjected to sucrose tolerance tests and the corresponding blood sugar, amino acid nitrogen, and urea nitrogen was determined at the fasting, one-hour, two-hour, and three-hour intervals following the administration of the sugar.

Analysis of the data in Table I demonstrates the fact that alimentary sucrose also depresses the postabsorptive level of amino acid nitrogen and urea nitrogen. In general, the results are similar to those secured with glucose. Thus the average maximum decrease in amino acid nitrogen re-

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TABLE 1

THE INFLUENCE OF ALIMENTARY SUCROSE ON THE AMINO ACID NITROGEN AND UREA NITROGEN CONTENT OF THE BLOOD OF PATIENTS

CASE NO.	BLOOD SUGAR MG./100 C.C.				URINE AMINO ACID N. MG./100 C.C.				UREA N. MG./100 C.C.				DIAGNOSIS	
	F.	1 HR.	2 HR.	3 HR.	SUGAR	F.	1 HR.	2 HR.	3 HR.	F.	1 HR.	2 HR.		3 HR.
1	105	130	99	99	Neg.	7.0	6.7	6.8	6.3	11.5	10.6	10.2	9.7	Pluriglandular endocrine disorder Diffuse colloid goiter; syphilis Syphilitic cardiovascular disease; aortic insufficiency
2	100	115	111	100	Neg.	5.6	5.4	5.3	5.2	5.9	5.7	5.5	5.0	
3	95	98	103	91	Neg.	6.8	6.7		7.0	21.0	19.7	20.0	18.2	
4	100	125	111	91	Neg.	6.7	6.1	5.8	6.0	12.5	11.5	10.7	10.9	Idiopathic epilepsy Syphilis; dental caries Pylorospasm Cellulitis, left leg Hypertensive cardiovascular disease Left hemianesthesia
5	91	114	103	81	Neg.	5.9	5.6	5.4	5.4	8.1	7.8	7.0	7.2	
6	91	118	74	78	Neg.	5.2	5.2	5.2	5.1	7.5	5.9	5.5	6.0	
7	115	179	114	95	Neg.	6.5	5.8	5.8	5.6	8.0	8.5	7.5	6.9	Hypertensive cardiovascular disease Left hemianesthesia
8	98	160	136	105	Neg.	6.6	6.3	6.3	5.8	6.5	6.1	5.6	5.3	
9	100	139	83	87	Neg.	6.7	6.7	6.1	6.2	16.7	16.2	15.5	14.5	
10	100	137	95	105	Neg.	5.6	5.5	5.3	4.9	9.1	9.1	8.9	9.1	Bronchitis; toxic erythema Hallux valgus deformity; lymphangitis Acute infectious arthritis, right hip Lymphangitis; infectious arthritis of knee Chronic multiple arthritis; infected tonsils Syphilitic cardiovascular disease; nephritis Tertiary syphilis; hypertensive cardiovascular disease
11	95	95	91	98	Neg.	5.6	5.4	5.0	4.9	6.3	5.6	5.5	5.2	
12	100	157	145	133	Neg.					13.3	13.4	12.4	11.3	
13	95	154	112	95	Neg.	6.0	6.0	5.7	5.7	18.8	16.7	16.7	16.5	Lymphangitis; infectious arthritis of knee Chronic multiple arthritis; infected tonsils Syphilitic cardiovascular disease; nephritis Tertiary syphilis; hypertensive cardiovascular disease
14	88	95	96	91	Tr.	6.6	6.1	5.7	5.7	13.7	13.2	12.6	12.4	
15	100	133	133	130	Neg.	6.2	5.7	5.9	5.7	10.0	9.1	8.5	8.2	
16	95	98	103	91	Neg.	6.8	6.7	7.0	7.0	20.9	19.7	20.0	18.9	Tenosynovitis right shoulder; otitis media Typhoid fever; syphilis Chronic multiple arthritis; goiter; caries Toxic polyneuritis; hyperkeratosis of the feet
17	100	200	204	147	Neg.	7.2	6.9	6.5	6.5	20.6	20.0	18.9	18.0	
18	100	167	208	181	Neg.	5.2	5.2	4.5	4.8	17.7	18.8	17.2	16.8	
19	105	190	133	91	Neg.	6.1	5.9	5.8	5.4	6.0	5.9	6.3	5.6	Arthritis; hypertensive cardiovascular disease Multiple infectious arthritis; infected tonsil Acute pulmonary tuberculosis; pleurisy Diabetes mellitus; bilateral cataracts Diabetes mellitus; arteriosclerotic cardiovascular disease
20	100	175	142	136	+	6.5	6.1	5.8	6.1	11.7	10.7	9.9	10.1	
21	97	163	182	142	Neg.	6.6	6.1	6.0	6.4	7.5	7.2	7.5	7.1	
22	108	235	167	145	Neg.	7.0	6.4	6.4	6.0	10.7	10.0	8.9	8.8	Multiple infectious arthritis; infected tonsil Acute pulmonary tuberculosis; pleurisy Diabetes mellitus; bilateral cataracts Diabetes mellitus; arteriosclerotic cardiovascular disease
23	91	200	192	167	Neg.	5.2	5.2	5.0	5.0	8.8	8.7	7.8	7.9	
24	91	222	142	80	Neg.	5.1	4.8	4.8	5.0	8.7	8.7	8.0	7.5	
25	118	238	222	187	++	7.0	6.5	7.0	7.1	12.5	11.6	11.5	12.5	Diabetes mellitus; arteriosclerotic cardiovascular disease
26	118	235	159	99	++	5.3	5.0	4.8	4.9	16.3	16.0	14.4	15.3	
Av.	99.9	159	132	117.2		6.2	5.9	5.7	5.75	11.9	11.4	10.86	10.95	
Av. maximum decrease (per cent)									8.07				8.74	

sulting from the ingestion of sucrose was 8.07 per cent as compared with a 11.78 per cent decrease in the same constituent produced by a similar quantity of dextrose. The average maximum decrease in urea nitrogen was 8.74 per cent as compared with a 13.04 per cent decrease with glucose. Thus alimentary sucrose seems to produce a somewhat smaller reduction in these nitrogen constituents of the blood than does an equivalent quantity of dextrose.

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## ON CHRONIC HYPERTENSION OF NERVOUS ORIGIN\*

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EVIDENCE which has been accumulating within the last ten years has definitely proved that blood pressure is self-regulated by a reflex apparatus governed by impulses taking origin in special pressure receptors. These are located (Fig. 1) in definite areas of the arterial system: the walls of the aorta, close to the heart, and the two swellings at the beginning of each of the two internal carotid arteries, known as the *carotid sinuses* (Fig. 2).

In the regions where receptors are located, the arterial walls are thinner and have a peculiar structure (fewer muscle fibers and more numerous elastic fibers) which make them more susceptible to changes in pressure. Deep in their adventitia they show rich nervous branchings which are connected with the pressure receptors. These, as shown by De Castro (1928), either keep a parallel direction to the very regularly arranged fibers of collagen (Fig. 3) or form spirals among them. Both dispositions predispose to change of form or position following changes in the degree of distention of the arterial walls.

Two nerves arise from the aortic receptors, the *aortic nerves*, whose development and distribution are better understood since Tello (1924) studied them. Another pair of nerves, the *carotid sinus nerves*, originate in the carotid sinus receptors and enter into the glossopharyngeus as it emerges from the skull.

By faradizing the central end of aortic or carotid sinus nerves, reflex heart retardation and fall of blood pressure are evoked†.

But what is more important than the results of artificially stimulating these nerves, is to know what are the effects exerted by the natural stimuli, playing continually upon the receptors from which they originate, i.e., by changes in arterial blood pressure. These have been demonstrated by different methods.

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†For this reason the name depressor nerve originated to designate the aortic nerves when these were the only known can no longer be used specifically for them.

TABLE 1

THE INFLUENCE OF ALIMENTARY SUCROSE ON THE AMINO ACID NITROGEN AND UREA NITROGEN CONTENT OF THE BLOOD OF PATIENTS

CASE NO.	BLOOD SUGAR MG./100 G.C.				URINE AMINO ACID N. MG./100 G.C.				URIC ACID N. MG./100 G.C.				DIAGNOSIS	
	F.	1 HR.	2 HR.	3 HR.	SUGAR	F.	1 HR.	2 HR.	3 HR.	F.	1 HR.	2 HR.		3 HR.
1	105	130	99	99	Neg.	7.0	6.7	6.8	6.3	11.5	10.6	10.2	9.7	Pluriglandular endocrine disorder
2	100	145	111	100	Neg.	5.6	5.4	5.3	5.2	5.9	5.7	5.5	5.0	Diffuse colloid goiter; syphilis
3	95	98	103	91	Neg.	6.8	6.7		7.0	21.0	19.7	20.0	18.2	Syphilitic cardiovascular disease; aortic insufficiency
4	100	125	111	91	Neg.	6.7	6.1	5.8	6.0	12.5	11.5	10.7	10.9	Idiopathic epilepsy
5	94	148	103	81	Neg.	5.9	5.6	5.4	5.4	8.1	7.8	7.0	7.2	Syphilis; dental caries
6	91	118	74	78	Neg.	5.2	5.2	5.2	5.1	7.5	5.9	5.5	6.0	Pylorospasm
7	115	179	114	95	Neg.	6.5	5.8	5.8	5.6	8.0	8.5	7.5	6.9	Ceclinitis, left leg
8	98	160	136	105	Neg.	6.6	6.3	6.3	5.8	6.5	6.1	5.6	5.3	Hypertensive cardiovascular disease
9	100	139	83	87	Neg.	6.7	6.7	6.1	6.2	16.7	16.2	15.5	14.5	Left hemianesthesia
10	100	137	95	105	Neg.	5.6	5.5	5.3	4.9	9.1	9.1	8.9	9.1	Bronchitis; toxic erythemia
11	95	95	91	98	Neg.	5.6	5.4	5.0	4.9	6.3	5.6	5.5	5.2	Hallux valgus deformity; lymphangitis
12	100	157	145	133	Neg.					13.3	13.4	12.4	11.3	Acute infectious arthritis; right hip
13	95	154	112	95	Neg.	6.0	6.0	5.7	5.7	13.8	16.7	16.7	16.5	Lymphangitis; infectious arthritis of knee
14	88	95	96	91	Tr.	6.6	6.1	5.7	5.7	13.7	13.2	12.6	12.4	Chronic multiple arthritis; infected tonsils
15	100	133	133	130	Neg.	6.2	5.7	5.9	5.7	10.0	9.1	8.5	8.2	Syphilitic cardiovascular disease; nephritis
16	95	98	103	91	Neg.	6.8	6.7	7.0	7.0	20.9	19.7	20.0	18.9	Tertiary syphilis; hypertensive cardiovascular disease
17	100	200	204	147	Neg.	7.2	6.8	6.5	6.5	20.6	20.0	18.8	18.0	lar disease
18	100	167	208	184	Neg.	5.2	5.2	4.5	4.8	17.7	18.8	17.2	16.8	Tenosynovitis right shoulder; otitis media
19	105	190	133	91	Neg.	6.1	5.9	5.8	5.4	6.0	5.9	6.3	5.6	Typhoid fever; syphilis
20	100	175	142	136	+	6.5	6.1	5.8	6.1	11.7	10.7	9.9	10.1	Chronic multiple arthritis; goiter; earies
21	97	163	182	142	Neg.	6.6	6.1	6.0	6.4	7.5	7.2	7.5	7.1	Toxic polyneuritis; hyperkeratosis of the feet
22	108	235	167	145	Neg.	7.0	6.4	6.4	6.0	10.7	10.0	8.9	8.8	Arthritis; hypertensive cardiovascular disease
23	91	200	192	167	Neg.	5.2	5.2	5.0	5.0	9.8	8.7	7.8	7.9	Multiple infectious arthritis; infected tonsil
24	91	222	142	80	Neg.	5.1	4.8	4.8	5.0	8.7	8.7	8.0	7.5	Acute pulmonary tuberculosis; pleurisy
25	118	238	222	187	++	7.0	6.5	7.0	7.1	12.5	11.6	11.5	12.5	Diabetes mellitus; bilateral cataracts
26	118	235	159	99	++	5.3	5.0	4.8	4.9	16.3	16.0	14.4	15.3	Diabetes mellitus; arteriosclerotic cardiovascular disease
Av.	99.9	159	132	117.2		6.2	5.9	5.7	5.75	11.9	11.4	10.86	10.95	
Av. minimum decrease (per cent)									8.07				8.74	

changes in the general blood pressure; (2) that the reflexes thus evoked (see Fig. 4) are mediated, mainly through changes in the vagal cardioinhibitory tonus and the general vasoconstrictor tonus, and secondarily upon the general vasomotor tonus and the cardioacceleratory tonus.

By means of these cardiac and vascular reflexes, both the *aortic and carotid sinus nerves maintain cardiac activity and blood pressure within limits of great constancy*. Under normal conditions their antagonizing effects may be as great as to counterbalance the general rise of blood pressure evoked by splanchnic stimulation (Fig. 5, A)

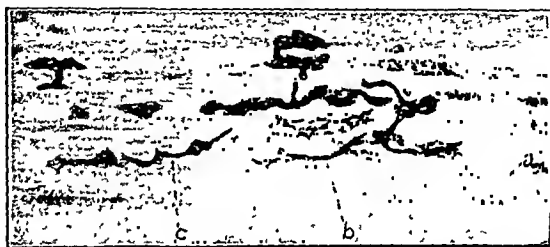


Fig. 3—The sensitive receptors (b and c) from which the carotid sinus nerves are issued, a, elastic layer lying deeply in the adventitia (From De Castro, 1928)

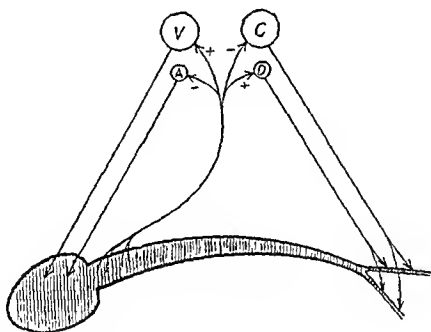


Fig. 4—A sketch of the circulatory reflexes originating in the pressure receptors V, vagus nerve, C, vasoconstrictors, D, vasodilators, +, excitation, -, inhibition (From Koch, 1931)

When the aortic nerves have been previously severed and the carotid sinus nerves have been functionally excluded by clamping of the two common carotids, the blood pressure rises and the heart beats are at a higher rate (Fig. 5, B). But if then the splanchnic nerve is stimulated under the same conditions as before, the blood pressure curve is considerably higher than when both sets of nerves are intact (Fig. 5, B). This is because vasoconstrictor-

tion is left free to come into play without being antagonized by the compensatory reflexes.

The self-regulation exerted by this apparatus gains in accuracy on account of its being formed by four units with their origins located in different parts of the arterial system. When one of these units becomes activated, the others react at once to counterbalance the reactions produced by the former. This antagonistic action of the other units made it at first difficult to demonstrate the different tonus effects individually exerted by each of them.

Readers interested in the development and details of the problem are referred to three excellent monographs, written by Hering (1927), by Koeh (1931), and by Heymans and collaborators (1932).

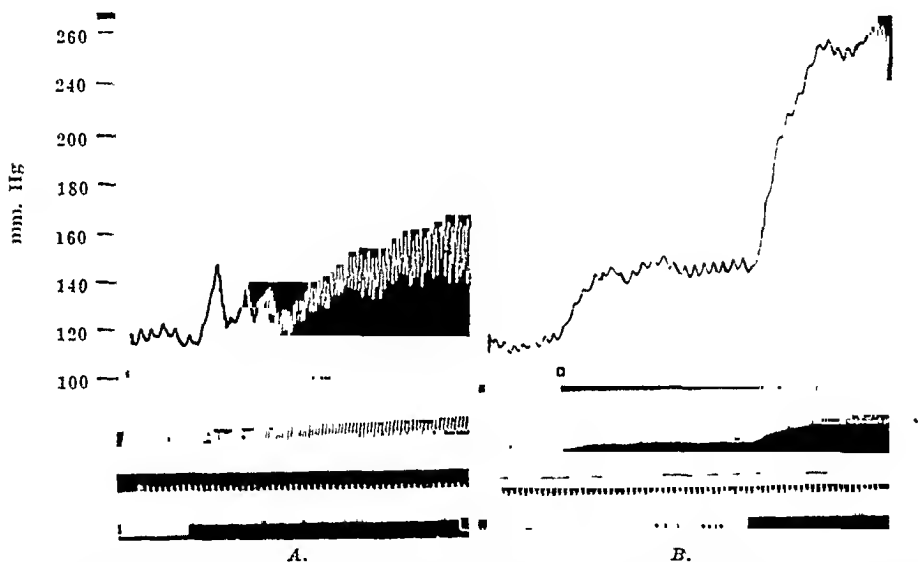


Fig. 5.—Effects of faradizing the peripheral end of the splanchnic nerve. A, when both aortic and carotid sinus nerves are intact. B, after the two aortic nerves have been severed and the two common carotids have been clamped at  $\alpha$ . (From Izquierdo, 1930.)

#### POSSIBLE SIGNIFICANCE OF THESE REACTIONS

Now, let us turn to the question of the relations it might have with the development of hypertension. *Does there exist, in animals or in man, some form of chronic hypertension due to reduced or even absent function of this self-regulating mechanism?*

Experiments in animals have provided a satisfactory answer to this question. Animals cannot live with the high blood pressure produced by the total elimination of the four presso-sensitive nerves. But if the nerves of one side are first removed and then those on the other, they survive, especially if important amounts of blood are drawn when the second operation is performed. Rabbits operated upon by Koeh and Mies (1929) lived with hypertensions of 150 to 180 mm. Hg (their normal blood pressure being between 90 and 100 mm. Hg) for more than a year and a half, which is about a fourth of their

normal lifetime. I have seen dogs in which high blood pressure (between 200 and 250 mm. Hg) persisted some months after elimination of their two sets of presso-sensitive nerves.

But is hypertension of this kind found in man? Is there any morbid process capable of suppressing, as in the experiments, all the four presso-sensitive nerves? Although it seems that this may happen, at least in certain forms of beriberi polyneuritis (see Koeh's monograph), it is not likely that the problem of human chronic hypertension should be referred to as an alteration of this kind.

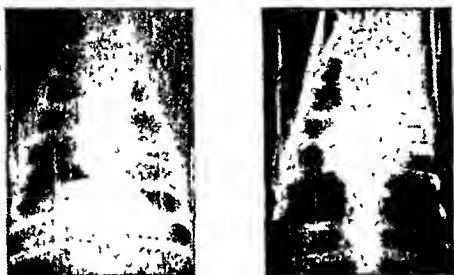


Fig 6—Cardiac shadows in the rabbit, in normal condition and twelve days after total suppression of the four presso-sensitive nerves (Plates from Hammer and Miles, 1929)

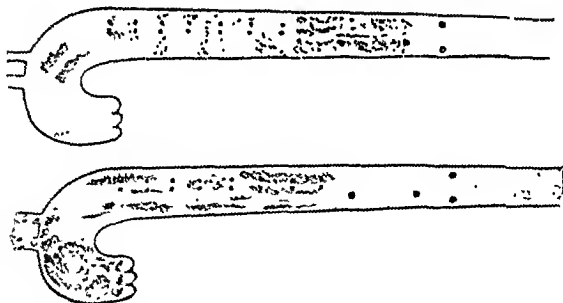


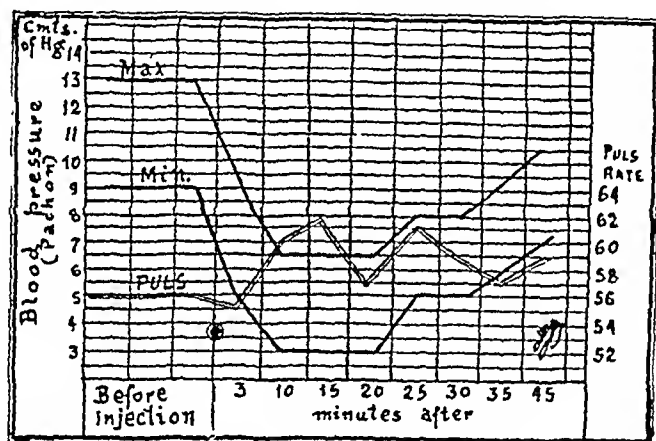
Fig 7—Distribution of the sclerotic lesions developed in the aortas of two rabbits after two months of chronic hypertension (140 mm Hg) (From Nordmann, 1929)

It is more probable that in man the morbid lesion must be located in the receptors themselves, primarily or secondarily altered by previous changes in the arterial walls, where they are located.

It has been known for more than twenty years (Hirsch and Thorspecken, 1912), that experimental hypertension, produced and maintained by repeated injections of adrenalin, produces at length calcareous infiltrations and points of lesser resistance in the aortic walls. Furthermore, in animals with chronic hypertension from suppression of all the presso-sensitive nerves, radioecopy has shown during life, and postmortem has confirmed after death, that the

heart becomes at first dilated (Fig. 6) and then hypertrophied; that the aorta is also distended and that in this and other arteries the well-known lesions of arteriosclerosis (Fig. 7) are present (Hammer and Mies, 1929; Nordmann, 1929; Mies, 1930). There is no possible doubt about this: hypertension may lead by itself to the production of arteriosclerosis, probably as the result of strain which a continuous exaggerated distention puts upon the circulation within the arterial walls.

According to current views among clinicians this same order of events would take place in man. For them, exaggeration of the arterial tone constitutes the initial trouble, which according to Volhard and his school would be the result of vasoconstrictor substances taking their origin from circulatory and renal deficiencies. But one might suspect, as well, that this might be the result of a primary functional deficiency of the reflex apparatus which regulates blood pressure. This seems to be supported by the fact that in some



© Injection of 1 c.c. of adrenalin i.p. 1.00  
in the vein.

Fig. 8.—Changes in blood pressure and in heart rate, determined by strong reflexes evoked in man by an injection of adrenalin. (From Izquierdo, 1921)

persons hypertension may go for years without leading to the production of cardiovascular lesions. But as is also known, the opposite is true, i. e., some individuals with arteriosclerosis never develop hypertension. It is therefore evident that to solve the problem, additional information is needed upon the functional condition of the units integrating the self-regulating mechanism.

It is desirable to develop some convenient method for testing clinically the functional responsiveness of the whole self-regulating apparatus, to general rises in the general blood pressure, as when adrenalin is injected. To begin with, the normal type of reaction must be found. I have seen that the rises of blood pressure thus produced in persons with highly reactive nerves evoke such powerful reflex actions as to completely counterbalance and even reverse (Fig. 8) the direct pressure-raising action of the drug. Such results are but a mere duplication of those observed in the laboratory. In persons with lowered reactivity of their pressor-sensitive nerves, the same doses of adrenalin are expected to produce much higher rises of pressure than when

conditions are normal. Of course, such a test will be permissible only in individuals in whom no vascular ruptures might be expected.

The functional reactivity of the two carotid sinuses has been tested by compressing them from outside. The slowing of the heart thus produced was believed, since the investigations of Tsehermack, to be due to a mechanical stimulation of the vagus. But Hering proved, first, that this is not stimulated by pressure, and second, that positive results are obtained only when pressure is exerted exactly upon the point where the carotid bifurcates. Compression of



Fig 9—The two Hering's points for the carotid compression (From Hering 1927)

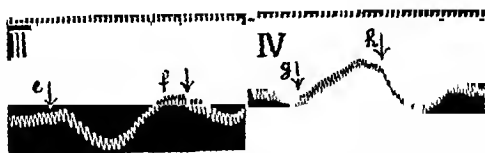


Fig 10—The effects evoked in man by compression of the carotid artery. In III the compression is exerted upon the two carotid sinuses between *c* and *f*. In IV both common carotids are compressed from *g* to *h*. (From Regniers 1930)

point *I* in Fig 9 where the carotid sinus is felt to beat evokes in man a double reflex (Fig 10) which slows the heart and makes the blood pressure fall. Nevertheless it has been shown that the starting point for these reflexes is not in the receptors themselves but in the compressed carotid sinus nerves, particularly if pressure is more effectively applied upon them through an artery with indurated walls. The method is therefore, unsuited for getting information on the receptors themselves.

Another method also developed by Hering which is but a particular application of the occlusion method formerly referred to, is to be preferred



for this purpose. Pressure is exerted with the tips of three or four fingers upon the middle portion of the common carotid (point *II* in Fig. 9). Thus the carotid sinus becomes more or less emptied of its blood and its receptors cease to be excited by distention of the wall: a double reflex (Fig. 10, *TV*), both of tachycardia and hypertension, ensues. By discontinuing pressure and thus allowing the carotid sinus walls to redistend, with blood rushing in, the two opposite reflexes are elicited (Fig. 10, *IV*). The results are especially marked when both carotids are compressed and decompressed at the same time.

Both Regniers (1930) and Mies (1932) report having obtained very interesting results by using this method. In Regniers' patients with essential hypertension and no cardiovascular lesions, reopening of the carotid never produced either the vagal or the hypertensive reflexes. In the seventeen individuals with essential hypertension observed by Mies, the possibilities of renal lesions were ruled out by submitting them to adequate functional tests. In them, neither carotid compression nor decompression produced cardiac or vasomotor reflexes. In view of all these new data, the existence of a primary and permanent hypertension, due to functional deficiency of the nervous regulatory apparatus, seems to be on its way toward recognition.

Observations have also been reported which show that sclerosis of the arterial walls at the points where the receptors lie, may lead to elimination of the self-regulating mechanism (Hering, 1927). This would either create hypertension, or if hypertension is already present, convert it into an irreparable process.

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COLINA NUM 367

## AN ABRIDGED KEY TO THE SPECIES OF PATHOGENIC FUNGI\*

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THIS key is a companion to 'An Abridged Key to the Genera of Pathogenic Fungi' which appeared in this JOURNAL† A synopsis for most of the pathogenic species of the genus is given It must be borne in mind that these organisms vary in their morphology on different culture mediums, and that they may vary on the same sort of medium

In the instances of the species of the saprophytes, the more common members are keyed, but summary is given

Detailed literature should be consulted for the study of the Dermatophytes

The Genera in this key are numbered as in the *key to the genera*

### 1 KEY TO THE SPECIES OF THE GENUS EPIDERMOPHYTON

The better known species of this genus may be recognized as follows

Lemon yellow growth on Sabouraud's agar

#### 1 *E. cruris*

Pale rose

#### *E. perneti*

Deep red

#### *E. rubrum*

The less known species which have been described are

*E. interdigitale*, *E. lanosum*, *E. nivenum*, *E. plumizoniforme*, and *E. salmonaceum*

#### 1 *Epidermophyton cruris* Castellani, 1905

Examination of the skin in hydroxide shows an abundance of filaments 4-5 $\mu$  in diameter, composed of quadrangular cells joined end to end and disassociating readily These filaments are developed horizontally in hardened areas of the epidermis The deeper layers of the skin are not invaded

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†Shaw Frederick W J LAB & Clin Med 20 112 1931

On culture mediums, growth is slow. The color is lemon yellow, dry and powdery, but becomes rapidly downy and white, by pleomorphism. Microscopic examination reveals the characteristic pluriseptated spindles.

Pathogenesis: Eczema marginatum or Tinea eruris.

## 2. KEY TO THE SPECIES OF THE GENUS TRICHOPHYTON

The more common species may be classified as follows:

### Endothrix

Cultures acuminate

Growth white

1. *T. acuminatum*

Growth violet

2. *T. violaceum*

Cultures crateriform

Yellow center with white periphery

3. *T. crateriforme*

Cultures crateriform and creased; white

4. *T. plicatile*

Cultures cerebriform

5. *T. cerebriforme*

### Ectothrix

Small spores ( $3\mu$ ); growth rapid

Growth starlike, powdery surface, plaster-like; frequently coiled bodies

Gypseum group

Growth snowflake-like, downy; coils and spindle spores lacking

Nivenum group

Large spores ( $5-8\mu$ )

Megaspore group

1. *Trichophyton acuminatum* Blanchard, 1895.

When the hair is examined in hydroxide, short and very fragile mycelial elements, which dissociate into round, oval or cylindrical spores of from 5 to  $7\mu$  in diameter, are seen.

On culture mediums the growth begins in the form of a small hemispherical cone, surmounted with three or four fimbriated, pinnate points. Older cultures may become lacunar.

Pathogenesis: Ringworm of hair, skin, nails.

2. *Trichophyton violaceum* Bodin, 1902.

This endothrix fungus was discovered by Sabouraud in cases of dry tinea of the scalp and beard. On culture it grows with the form of a slight disk, acuminate at the center. The growth is thin, moist, and pale brown or gamboge in color at the beginning; later it becomes larger. Small riddled furrows are formed, and in about three weeks the culture becomes a violet color. This color is lost after a number of transplants. No spores are formed in cultures.

### 3 *Trichophyton crateriforme* Sabourand, 1902

When examined in hydroxide, the hair is seen to be filled with simple filaments, formed by a series of quadrangular spores of 4 to 5 $\mu$  in length giving the appearance of a ladder. Growth on maltose medium begins as a white growth, which extends until the border is raised. The consistency of the colony is velvety, at first, later becoming dry and powdery. As growth continues, the color becomes creamy.

Pathogenesis Ringworm of the scalp, herpes circinata of the smooth skin, ringworm of the nails and dry trichophytosis of the eyelashes.

### 4 *Trichophyton plicatile* Sabourand 1909

This fungus may be found around the hair. Cultures resemble those of *T. crateriforme*. They are white and powdery with their surfaces folded at the center, the periphery presents a powdery zone.

Pathogenesis Dry and wet trichophytosis of the beard.

### 5 *Trichophyton cerebriiforme* Sabourand 1893 1909

The skin when examined in hydroxide resembles that of *T. crateriforme*. On culture this fungus may be distinguished from *T. crateriforme* by the chuffon appearance of its surface and its color which becomes cream in old cultures. The periphery of the colony shows powdery like rays distinct and unequal, while that of *T. crateriforme* is a powdery like annule.

Pathogenesis Herpes circinata ringworm of the scalp and beard.

## 3 KEY TO THE SPECIES OF THE GENUS ACHORION

Yellowish colonies slight duvet in the center, the remainder smooth

### 1 *A. gypseum*

Colonies uniformly white with duvet

### 2 *A. quinckeianum*

Colonies light yellow cerebriiform

### 3 *A. schoenleini*

### 1 *Achorion gypseum* Bodin 1907

Colony flat yellowish with a little tuft of white down in the center and a white border. Isolated from a case of favus.

### 2 *Achorion quinckeianum* Zopf 1890

In the lesions the mycelial filaments are abundant and are broken up into small segments which constitute the spores. On culture medium the growth is uniformly white with a duvet and a wrinkled center. The organs of reproduction appear about the fourth day and are of the Acladium type. The cause of Mouse Favus, transmissible to man.

### 3 *Achorion schoenleini* Remak 1845

The fungus may be demonstrated in either the scutulum or hair and appears as rounded cells oval cells and short and long filaments. Colonies are at first yellowish and waxy becoming much wrinkled with age. Some strains develop whitish aerial hyphae. This fungus is extremely pleomorphic. The cause of favus.

4. KEY TO THE SPECIES OF THE GENUS MICROSPORON

Radial furrows; small central, elevated knob.

1. *M. audouini*

No furrows

Central portion of colony flat

2. *M. lanosum*

Small central knob of light yellow

3. *M. felineum*

1. *Microsporon audouini* Gruby, 1843.

Clarified with hydroxide or other fluids, the hair presents, under the microscope, a multitude of small spores 2 to 3 $\mu$  in diameter, polyhedral and doubly contoured. Hyphae on inside, spores on outside of hairs. The colonies are snow white with a small central knob elevated above the surface, and containing from four to six deep clefts. The whole surface of the colony is covered with a fine duvet. Cultures grow slowly. Causes ringworm of scalp.

2. *Microsporon lanosum* Sabouraud, 1907.

Hairs in hydroxide similar to *M. audouini*. Colonies show a central flat elevated ring of aerial hyphae; no folding; woolly surface; become reddish in the center, when spindle spores are formed. Causes ringworm of the scalp.

3. *Microsporon felineum* Fox and Blaxall, 1896.

Rapid growth on culture medium. Colony regular in form, with a small central disk of a yellowish color. The periphery is composed of a fine radiating duvet.

Pathogenesis: Tinea capitis very often accompanied by cutaneous lesions.

5. KEY TO THE SPECIES OF THE GENUS ENDODERMOPHYTON

Cultures become black

Rapidly

1. *E. mansonii*

Slowly

2. *E. concentricum*

Cultures red

3. *E. indicum*

Cultures light yellow

4. *E. tropicale*

1. *Endodermophyton mansonii* Castellani, 1914.

Direct examination of the scales of the skin in hydroxide shows a network of mycelial filaments of a greenish yellow color, and are composed of oval or rectangular elements of unequal dimensions, or of chains of spores. Slow growing; at first amber in color, later black; no duvet. Found in *Tinea imbricata*.

2. *Endodermophyton concentricum* R. Blanchard, 1895.

Differs from the above by its cultures which slowly turn black. Found in the same conditions.

3. *Endodermophyton indicum* Castellani, 1911.

Differs from the above by its yellow cultures and duvet. Later the growth becomes red.

4 *Endodermophyton tropicale* Castellani, 1914

Examination of the scales of the skin similar to the above Colonies wrinkled, slight amber color, becoming deeper with age, duvet usually absent Found in the same condition as the above species

6 KEY TO THE SPECIES OF THE GENUS *MALASSEZIA*

Causes a brown eruption on white skin

1 *M. furfur*

Causes a reddish yellow eruption on white skin

2 *M. tropica*1 *Malassezia furfur* Ch Robin, 1852

When the skin scrapings are examined in hydroxide solution, the fungus is seen in abundance either in the form of filaments or of rounded spores The filaments measure about  $3\mu$  in diameter, are unbranched, flexuous, continuous or septate The spores are from 3 to  $5\mu$  in diameter, generally in masses of from 15 to 30 elements Said to have been cultivated on glycerinated gelatin Causes pityriasis versicolor in man

2 *Malassezia tropica* Castellani, 1908

In the epidermis this fungus produces a thick mycelium with irregular contractions Causes a reddish yellow eruption on the white skin and a light yellow eruption on the dark skin Causes Tinea flava

7 KEY TO THE SPECIES OF THE GENUS *TRICHOSPORUM*

Nodules on hair polyhedral

3-4  $\mu$  in diameter

1 *T. beigeli*

5-8  $\mu$  in diameter

2 *T. hortai*

12-14  $\mu$  in diameter

3 *T. giganteum*1 *Trichosporum beigeli* Rabenhorst, 1867

On the hair this fungus produces irregular nodes which under the microscope, are seen to be made up of elements about 3 to  $4\mu$  in length In culture, rounded cells of about  $3\mu$  are obtained, which elongate into septated and branched filaments Chlamydospores are present in old cultures These are thick and refractive isolated situated intercalated or terminal They may be as large as  $12\mu$  in diameter Causes Trichosporosis of the beard

2 *Trichosporum hortai* Brumpt 1913

The nodules on the hair are polyhedral and are from 5 to  $8\mu$  in diameter Growth on culture medium is slow, greenish in color, later brownish black Strongly attached to the substratum Grows on potato Causes Piedra

3 *Trichosporum giganteum* Behrend, 1890

Direct examination of the hair shows polygonal cells from 12 to  $14\mu$  in diameter forming the nodes Except at the epidermal margin, the hair is not much altered by the invading fungus Examination of the cultures re

veals filaments from 10 to  $60\mu$  in length, by 1 to  $4\mu$  in width, frequently branched, septate. The spores are of variable size and shape, in masses or in chains, 2 to  $12\mu$  in diameter. Causes Piedra.

#### 8. KEY TO THE SPECIES OF THE GENUS *INDIELLA*

Grains hard and beaushaped

1. *I. mansonii*

Grains soft, composed of mycelium in coiled masses

2. *I. reynieri*

1. *Indiella mansonii* Brumpt, 1906.

This fungus is known only in the parasitic stage. The mycelium is white, delicate when young, and measures about 1 to  $2\mu$ , but some elements may be 15 to  $20\mu$ . The old filaments become irregular, 3 to  $5\mu$  in diameter; the septa vary from 5 to  $10\mu$ . Chlamydospores are generally terminal, rarely intercalated, of from 5 to  $12\mu$  in diameter, generally spherical, unicellular, at times segmented. The granules are from  $\frac{1}{6}$  to  $\frac{1}{4}$  mm., flat. Causes mycetoma.

2. *Indiella reynieri* Brumpt, 1906.

Mycelium white, living as a parasite in man. The young mycelium is delicate, of a diameter of 1 to  $5\mu$ ; the septa are from 10 to  $15\mu$  in length. The peripheral filaments are irregular, moniliform, acquire a diameter of from 4 to  $5\mu$ . Most of the filaments present chlamydospores at the ends, which are often divided into two or three pieces. The filaments always remain rolled up or twisted, and form a characteristic granule, which is round when young, but twisted and cordlike when old. The granules do not exceed 1 mm. in diameter. This organism causes white grain mycetoma.

#### 9. KEY TO THE SPECIES OF THE GENUS *MADURELLA*

Numerous black sclerotia at periphery of cultures

1. *M. americana*

Sclerotia formed below the surface of culture medium

2. *M. mycetomi*

Sclerotia rarely produced; when present, on surface only

3. *M. tozeuri*

1. *Madurella americana* Gammel, Miskdjian, and Thather, 1926.

Black granules of about 1 mm. in diameter in masses of from 3 to 5 mm. Structures resembling chlamydospores may be seen after treatment with hydroxide. On culture the growth is at first white, then becomes more and more brown, which is followed by a diffusion of the pigment into the medium. This pigment is secreted from numerous sclerotia at the periphery of the culture. The cause of American mycetoma.

2. *Madurella mycetomi* Laveran, 1902.

Black granules, small, hard, rounded, verrucose. Growth on mediums grayish white, becoming yellowish, then blackening the medium. Oidia of from 2 to  $5\mu$ . Sclerotia sterile, black,  $\frac{1}{2}$  to 1 mm. The cause of mycetoma with black granules.

3 *Madurella tozzii* Nicolle and Pinoy, 1908

Black granules of amorphous structure, the granules being composed, often, of a mycelial ring enclosing degenerated cellular elements. The pigment is derived from the fungus in these masses. The growth on culture medium is at first white, becoming yellowish with age, followed by blackening of the medium. Causes mycetoma with black granules.

10 KEY TO THE SPECIES OF THE GENUS *BLASTOMYCOIDES*

In tissues with a thick mucous capsule

1 *B. histolytica*

Thick mucous capsule not present in tissues

Reproduce by budding

2 *B. dermatitidis*

Reproduce by endospores

3 *B. immutis*1 *Blastomycoides histolytica* Stoddard and Cutler, 1916

This organism appears in the tissues as round, budding cells with a thick capsule of mucoid material. In cultures no capsules are present, no mycelium is formed, no ascospores have been observed. Growth is slow, creamy, most abundant on potato and dextrose agar. No gas is formed from carbohydrates. The cause of infection of the brain and meninges.

2 *Blastomycoides dermatitidis* Gilchrist and Stokes, 1898

In the tissues the fungus occurs as a round or oval budding cell, with a thick and highly refractile wall. No endospores are present. Growth on culture medium may present three types and transitions between them. The one may resemble the growth of the tubercle bacillus, another the acladium and the third the cottony type. Yeastlike cells and branching, segmented hyphae may be seen on microscopic examination. This will vary with the age of the culture and other factors. This organism causes blastomycosis.

3 *Blastomycoides immutis* Bixford and Gilchrist, 1897

In the lesions, roundish bodies which vary from 3 to 80 $\mu$  in diameter, generally with a thick capsule (although the small forms may have a thin capsule) which is well defined. Within some of these structures are numerous spores. Reproduction by budding never occurs. On culture medium the fungus may be of two types, a yeastlike type, reproducing by budding, and a filamentous type. The colonies are round and somewhat elevated. Old cultures may show chlamydospores. The mycelium is septate and branched. An abundance of white aerial hyphae is formed, no conidia are present. The growth resembles that of *B. dermatitidis*, but differs microscopically in the production of large numbers of chlamydospores. This fungus produces coccidioidal granuloma.

11 KEY TO THE SPECIES OF THE GENUS *HISTOPLASMA*

Colonies asteroid

1 *H. stellata*

Colonies cerebriform

2 *H. rugosa*



1. *Hemispora stellata* Vuillemin, 1906.

In the lesions, direct examination reveals the mycelium in the form of white disks,  $\frac{1}{2}$  to  $2\frac{1}{2}$  mm. in diameter, sessile, covered with conidiophores designing stars on the surface. In culture, irregular growths are obtained with a stellate appearance. Isolated from osseous lesions and cold abscesses.

2. *Hemispora rugosa* Castellani, 1910.

This species produces cerebriform colonies. Isolated from sputum.

## 12. KEY TO THE SPECIES OF THE GENUS DEMATIUM

Conidia globose, spiny, in chains

*D. atro-purpureum*

Conidia pear-shaped, warty, in chains

*D. scabridum*

Conidia globose, smooth, short chains

*D. castaneae*

## 13. KEY TO THE SPECIES OF THE GENUS HAPLOGRAPHIUM

Conidia apiculate

*H. apiculatum*

Conidia elongated, elliptic

*H. atro-brunneum*

## 14. KEY TO THE SPECIES OF THE GENUS CLADOSPORIUM

Pathogenic

Associated with *Tinea nigra*

1. *C. mansonii*

Associated with ulcerating nodules

2. *C. penicillioides*

Saprophytes

Hyphae pale green

*C. epiphyllum*

Hyphae brown or olive

*C. herbarum*

1. *Cladosporium mansonii* Castellani, 1908.

In the skin the fungus appears as elongated filaments of from 18 to  $20\mu$  in length, by 2 to  $3\mu$  wide; septate, unbranched. The rounded spores are 5 to  $10\mu$ , and are often in masses. Black pigment is contained in all of these structures. On culture medium, colonies appear in from two to four days; black, becoming confluent, adherent to the substratum. Causes *Tinea nigra*.

2. *Cladosporium penicillioides* Preuss, 1848.

In the tissues the fungus appears in the form of ovoid or fusiform masses, 3 to  $4\mu$  in length. Cultures are readily obtained. These are chocolate brown, with a powdery surface; cerebriform colonies. This organism was isolated from an ulcerated nodular lesion of the leg.

15. THE GENUS *MONILIA*

For the classification of the species of this genus, the reader should consult special articles on this subject.

16. THE GENUS *ENTOMYCES*

The following species have been described, but the available data are insufficient for differentiation: *crateriforme*, *cruzi*, *molardi*, *pulmonalis* and *vuillemini*.

17. KEY TO THE SPECIES OF THE GENUS *RHIZOMUCOR*

*Columella* piriform

1. *R. parasiticus*

*Columella* spherical

2. *R. septatus*

1. *Rhizomucor parasiticus* Lucet and Costantin, 1900.

Colonies grayish to yellowish brown. Sporangiophores 1 to 2 mm. in length, ramified, rarely branched a second time, generally in clusters; sporangia 35 to 80 $\mu$  with a membrane bordered with fine crystalline needles; ovoid, piriform, hardened, slightly brownish columella 30 to 70 $\mu$  in height by 25 to 55 $\mu$  in width; lateral sporangia smaller. Spores round or oval, 4 by 2.5 $\mu$ . Zygosporangia absent. Isolated from sputum in pseudotuberculosis.

2. *Rhizomucor septatus* von Bezold, 1889.

Sporangiophores brownish, ramified, in clusters, average diameter 10 $\mu$ ; small rhizoids at the base; secondary sporangiophores 3 to 4 $\mu$ , short with transverse septa at the point of ramification. Sporangia pale grayish brown, spherical, with a transparent membrane, 32 $\mu$  in diameter. Columella spherical or slightly ovoid, brown, 25 $\mu$  in diameter. Spores spherical or oval, thin, yellow or brownish. This organism has not been cultivated. Isolated from the external auditory canal.

18. THE GENUS *RHIZOPUS*

*Rhizopus niger* Ciaglinski and Hewelke, 1893.

Stolons branched, with numerous rhizoids, forms a snow white mat. Upright sporangiophored pedicels, straight, fasciculated, terminated by spherical sporangium black at maturity. Columella at first cylindrical, 2 to 3 times longer than their width, enlarging later and presenting at maturity the appearance of a spherical cap; caving in after drying out of the sporangium and taking the form of an open umbrella. Spores oval, thin, gray. Zygosporangia heterothallic. Recovered from black tongue.

19. THE GENUS *ABSIDIA*

This genus contains no pathogens, at this time.

20. THE GENUS *MUCOR*

For the identification of species of this genus the reader should consult special monographs.

## 21. KEY TO THE SPECIES OF THE GENUS FUSARIUM

Conidia brownish to salmon color

Conidiophores simple or slightly branched

*F. orthocerus*

Conidiophores much branched; macroconidia not broader toward apex

Bluish-black sclerotia on potato

*F. oxysporum*

Sclerotia absent

*F. lini*

Conidia brown-white to golden brown, walls thick

*F. solani*

Conidia cream-buff to cinnamon

*F. caudatum*

## 22. KEY TO THE SPECIES OF THE GENUS ALTERNARIA

Conidia smooth

Colonies brown-green

1. *A. tenuis*

Colonies brown

*A. fasciculata*

Conidia rough

*A. humicola*

1. *Alternaria tenuis* Ness, 1817.

Colonies brown. Conidiophores short, septate, branched or unbranched, brown green. Conidia in chains, with cross and longitudinal walls (3 to 5 cross walls), constricted at the outer walls, olive green or brownish black, variable in size and shape, 30 to 35 $\mu$  by 15 $\mu$ . Usually saprophytic, but has been reported as pathogenic.

## 23. THE GENUS ACROTHECA

*Acrotheca pedrosi* Fonseca and Leao, 1923.

This is probably a blastomycoides. In the tissues are found ovoid or spherical blackish corpuscles within the giant cells. On culture, well-developed hyphae, 2.5 to 3.2 $\mu$  in diameter, with septa 17 to 24 $\mu$  in length; blastospores 5 to 8 $\mu$  by 3.5 $\mu$ ; conidia 1.5 by 4.5 $\mu$ . Causes a verrucose dermatitis.

## 24. THE GENUS PHIALOPHORA

*Phialophora verrucosa* Thaxter, 1915.

This organism is probably a blastomycoides. Occurs in the tissues in two forms: sclerotes and budding forms. The sclerotes are at first unicellular and vary from 8 to 15 $\mu$  in diameter; their walls are colored blackish brown. Septation soon follows with the production of sclerotes, pleuricellular, and budding forms which in turn may be transformed into sclerotes. Cultures are black, with a water soluble pigment. Branches of aerial hyphae end by sporogenous cells in the form of hollow cupola, which produce conidia 4 to 6 $\mu$  by 2 $\mu$ , and which remain agglomerated in masses of 4 to 20 elements, in the

form of globular false sponges, at first yellowish, then brown. Sclerites, similar to those in the tissues, are formed in ascitic gelatin and coagulated serum, and are terminal, septate. Conidial buds are produced. Causes a verrucose dermatitis.

## 25 KEY TO THE SPECIES OF THE GENUS *ACREMONIELLA*

Pathogenic

1 *A. perini*

Saprophytic

Conidia smooth

Pale ochre, finally olive

*A. fusca minor*

Black

*A. melanosperma*

Conidia rough, dark brown

*A. brevis*

1 *Acremonietta perini* Pollacci, 1923

Characterized by short conidiophores which give rise to a single brown conidium, finely echinulated. Isolated from sputum.

## 26 KEY TO THE SPECIES OF THE GENUS *CEPHALOSPORIUM*

Conidiophores branched, growth pure white

*C. cutipes*

Conidiophores unbranched, growth white, then rose

*C. acremonium*

*C. acremonium* has been reported as being pathogenic.

## 27 KEY TO THE SPECIES OF THE GENUS *TRICHODERMA*

Colonies white

*T. album*

Colonies green

Conidia globose

*T. lignorum*

Conidia ovoid to elliptic

*T. glaucum*

## 28 KEY TO THE SPECIES OF THE GENUS *ACREMONIUM*

Colonies finally orange

1 *A. potroni*

Colonies generally white

2 *A. niveum*

Colonies finally red

3 *A. muthuoni*

1 *Acremonium potroni* Vuillemin, 1911

Hypae septate, conidiophores numerous, elongated from 15 to 20 $\mu$  long, conidia ovoid, smooth, 4 to 5 $\mu$  long, 2 $\mu$  wide, pinkish, colonies at first white, then pink to orange. Isolated from papules.

2. *Acremonium niveum* Boucher, 1918.

Similar to *A. potroni*, colonies are generally white.

3. *Acremonium muthuoni* Fontoynt and Boucher, 1922.

Similar to *A. potroni*, but colonies become brick red.

## 29. THE GENUS CEPHALOTHECIUM

This genus is of no pathogenic importance.

## 30. THE GENUS DIPLOSPORIUM

*Diplosporium vaginae* Nannizzi, 1925.

Sporophore branched with a terminal conidia that is hyaline, oblong, bicellular, smooth, 15-20 $\mu$  by 5-6 $\mu$ . When the conidia are detached, they are replaced by another growing one, but remain joined together for a long time by means of a gelatinous membrane. Chlamydospores and isolated or clustered aleuriospores present. Isolated from a case of purulent vaginitis.

## 31. THE GENUS ALLANTOSPORA

Allantospora onychophila has been reported as being pathogenic, but there is doubt as to the classification of this organism.

## 32. KEY TO THE SPECIES OF THE GENUS CITROMYCES

Wehmer has described two species of this genus which produce citric acid from sugar. Thom places them with the Penicillia.

Conidia smooth

*C. glaber*

Conidia bristly

*C. pfefferianus*

## 33. KEY TO THE SPECIES OF THE GENUS ASPERGILLUS

This is a large genus. The monograph by Thom and Church should be consulted for detailed information. The more important species are given below.

Conidia white

*A. candidus*

Conidia colored

Green, bluish green, yellowish green or gray

Perithica readily produced

Perithicum embedded

Sterigmata apical

*A. fumigatus*

Sterigmata pleurogenous

*A. clavatus*

Perithicum not embedded

*A. glaucus*

Perithica not readily produced, if at all;  
conidiophore rough

*A flavus orzdae*

Blackish brown

*A calyptriatus luchuensis*

Light brown

*A wentii*A few species of the genus *Aspegillus* cause infection in man34 KEY TO THE SPECIES OF THE GENUS *STERIOMATOCYSTIS*

Conidial forms blackish brown

*S nigra*

Conidial forms green then gray to brown

*S nidulans*

The two species have been reported as pathogenic

35 KEY TO THE SPECIES OF THE GENUS *PENICILLIUM*

Colonies white

*P candidum*

Colonies white later yellow

*P wortmanni*

Colonies yellow or brown

*P brevicaulis*

Colonies red

*P roseum*

Colonies green

Glaucum italicum, olivaceum granulatum,

claviforme aurum purpurogenum, luteum

Consult "The Penicillia," by Thom, for details of this genus

36 KEY TO THE SPECIES OF THE GENUS *ACLADIUM*

Pathogenic

1 *A castellanii*

Saprophytic

*A densissimum*1 *Acladium castellanii* Pimov, 1916

Colonies consist of roundish masses, which may coalesce, covered with spicules which give the colony a prickly appearance, pseudocomidia of variable shape cylindrical pyriform or spherical, lateral, attenuated in size at their points of insertion 4 by 3 $\mu$ . Colonies on carrot and potato are white, on dextrose agar often amber, old cultures brown or black.

37 THE GENUS *ALLURISMA*

The following species have been reported, but in most instances the data are insufficient for differentiation *A arlougi* (probably an achorion), *A benignum*, *A lugdunense* and *A salmonicum*

38 THE GENUS *CONIOTHORIS*

But one species has been reported from *C hominis*. This fungus is known only in the parasitic state. It was found in a trichophid lesion of the forearm

## 39. KEY TO THE SPECIES OF THE GENUS GLENOSPORA

Aleuriospores 6 to  $10\mu$  by 5 to  $8\mu$

1. *G. grandavensis*

Aleuriospores 5 by  $3\mu$

2. *G. graphii*

1. *Glenospora grandavensis* Vuillemin, 1921.

Cultures blackish, covered with a white dust, formed from dissociated colorless pieces. The aleuriospores are 6 to  $10\mu$  by 5 to  $8\mu$ . Neither conidia nor endoconidia are present. Isolated from fetid bronchitis sputum.

2. *Glenospora graphii* Vuillemin, 1912.

Colonies at first colorless, later become brownish. The filaments are of the same color, branched, septate, 2 to  $3\mu$  in diameter. Aleuriospores are ovoid, thin walled, 5 by  $3\mu$ , become brown at maturity. These spores are isolated at the extremity of the branches. The straight filaments can effect a fasciculated appearance. The branches are arranged totally without order, nearly irregularly alternating and then subopposed or subverticulated. Causes otomycosis and keratomycosis.

## 40. KEY TO THE SPECIES OF THE GENUS SPOROTRICHUM

There is probably but one species pathogenic for man

1. *S. schenckii*

Nonpathogenic

Colonies red

*S. roseum*

Colonies white, cottony

*S. pruinosum*

1. *Sporotrichum schenckii* Hektoen and Perkins, 1900.

In the tissues and pus the fungus appears as small (3 to  $4\mu$  by 1.5 to  $3\mu$ ) spindle-shaped cells. In the pns treated with hydroxide these cells appear as highly refractile spores. They are gram-positive and generally occur within the polymorphonuclear leucocytes. No mycelium develops in the body.

Cultures present at first a soft, creamy, moist, shiny growth. As the culture ages, it becomes a light tan, deepening to a brown or black in most strains. The surface is wrinkled.

Microscopic examination of the growth from dextrose agar shows the organism to consist of branching hyphae from which ovoid conidia develop either from the sides of the hyphae or from the lateral or terminal filaments. Causes sporotrichosis.

## 41. THE GENUS MYCODERMA

This genus is of no pathogenic importance.

## 42. THE GENUS TORULA

There are no species of interest to the pathologist in this genus.

43 THE GENUS *CRYPTOCOCCUS**Cryptococcus hominis* Vuillemin, 1901

In the pus, oval bodies with a double contour. The elements are arranged in groups, each group embedded in an amorphous substance, surrounded with a capsule. Growth on culture medium white or slightly yellow. Yeastlike forms from culture, no mycelium, no ascus. Found in abscesses.

44 THE GENUS *DEBARYOMYCES*

Probably the most important pathogenic species of this genus is

*Debaryomyces hudelei* (de Benruann and Gougerot, 1909), Fonseca et al. 1922

Cells round, oval or elongated. 2 to 4  $\mu$  in diameter by 6 to 8  $\mu$  long. Ascus scanty, one spore, verrucose surface. Isolated from multiple abscesses.

45 THE GENUS *SACCHAROMYCES*

Monographs on the yeasts should be consulted for the species of this genus.

46 THE GENUS *WILLIA**Willia anomala* Hansen, 1904

Cells oval, small. Ascus contain two or four spores of the batlike type, dextrose and beet wort fermented but not maltose or saccharose, a membrane is formed in sugar liquid mediums (the membrane contains gas bubbles). Ethanol is produced from sugar mediums. Isolated from sputum.

## FERMENTATION AND GAS PRODUCTION BY B. COLI IN SIMPLE AND MIXED SUGARS\*

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## INTRODUCTION

THE choice of carbohydrate for modification of cow's milk in infant feeding has concerned pediatricians during the past several decades. Some clinicians have expressed themselves as being unable to detect significant differences among the various sugars. Yet one would expect, a priori, that some differences must exist even though so slight as to be overlooked in isolated or limited clinical observation. Other clinicians have definite preferences for various carbohydrates based either upon indefinite clinical impressions of superiority or upon the idea that certain carbohydrates are less readily attacked by the bacteria of the intestinal flora and thus cause less fermentation, gas and distention than do other sugars.

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The object of this study was to test this hypothesis by experiments in vitro. It seems reasonable to assume that single representatives of the intestinal flora will show differences in the fermentation of various carbohydrates in vitro similar to the differences which they show in the intestine. Although it is recognized that strict conclusions concerning in vivo processes cannot be drawn from in vitro experiments, such experiments are useful inasmuch as they may point to certain possibilities concerning in vivo processes.

Since *B. coli* is the main representative of the intestinal flora, strains of this organism were studied. They were obtained from the stools of two groups of infants, the subjects of a clinical comparison of cane sugar with a high dextrin carbohydrate.

The studies of Rettger and Cheplin,<sup>1</sup> Cruickshank,<sup>2</sup> Nissle,<sup>3</sup> and von Knorr<sup>4</sup> are of interest in relation to this work.

#### EXPERIMENTAL METHODS

The infants were fed a standard formula including milk and either cane sugar or carbohydrate C (high dextrin) as the sole additional sugar. The stools were tested at intervals of two weeks. Strains of *B. coli* were fished and tested separately.

The isolated strains were cultured in fermentation tubes. The basic medium consisted of 0.5 per cent peptone (Difeo) solution adjusted to pH 6.9-7.1. The carbohydrate was made up as a 10 per cent solution in 0.5 per cent peptone, sterilized by filtration through a Berkefeld, tested for sterility, and kept in the ice box. To prepare the media for the studies, sufficient of the 10 per cent carbohydrate solution was added (with aseptic precautions) to a definite quantity of sterile 0.5 per cent peptone solution to give the desired 0.5 per cent concentration of carbohydrate. Sterile Smith fermentation tubes were filled with 50 c.c. of sterile 0.5 per cent carbohydrate medium and incubated twenty-four hours for sterility.

Cultures of the *B. coli* were grown for eighteen hours on extract agar slants. The growth was washed with 2 c.c. of saline, and 0.1 c.c. of this suspension of bacteria inoculated into the Smith fermentation tubes containing the various carbohydrates. The tubes were incubated for twenty-four hours at 37° C. Smears were made from each tube and stained with gram stain to check the purity of the cultures.

The carbohydrate materials used in these fermentation studies included glucose, maltose, lactose, and carbohydrates A, B, and C. The last three are commercial preparations, A being in the nature of a syrup and B and C prepared from starch by partial hydrolysis. The chemical tests carried out before and after the bacterial growth included the amount of gas, pH, total acidity, and reducing actions both total and unfermentable.

The determinations were carried out as follows:

*Gas*.—The length of the gas column in the Smith tube was measured with a ruler with divisions in millimeters. The results are given as millimeters, and while perhaps not as accurate as might be wished for, are sufficiently so for the purposes in view.

*pH*.—Indicators and standard controls were used.

**Total Acidity**—To 10 c.c. of medium (before and after growth of organisms) in a 50 c.c. Erlenmeyer flask were added about 10 c.c. of distilled water and between 1.00 and 5.00 c.c. of accurately measured 0.1 N sulphuric or hydrochloric acid. The mixture was boiled for one to two minutes, cooled, and titrated with 0.1 N sodium hydroxide solution with phenolphthalein as indicator. The results are given as cubic centimeters of 0.1 N sodium hydroxide used, or tenths of milli equivalents of acid present in 10 c.c. of medium.

**Reducing Actions**—Total, unfermentable, and fermentable (by difference). The 1926 modification of Benedict's method was used.<sup>5</sup>

1. **Preparation of filtrates**—To 4 c.c. of medium were added 4 c.c. of distilled water, or (for unfermentable reductions) 4 c.c. of yeast suspension (i.e., one cake of Fleischmann's yeast suspended in 100 c.c. distilled water). The yeast fermentation was warmed somewhat (40° C.) and allowed to stand fifteen minutes. Then 1 c.c. of 10 per cent sodium tungstate solution and 1 c.c. of 0.7 N sulphuric acid were added and the mixture shaken, using a rubber stopper. After thirty minutes, the 10 c.c. mixture was filtered through 11 cm. Swedish paper.

2. **Dilution and analysis**—Because 2 c.c. of the filtrates of the different carbohydrate media were too strongly reducing to read against the glucose standard (0.2 mg. glucose in 2 c.c.), the following dilutions were used: glucose 2:25, maltose 1:8, lactose 1:8, A 1:4, B 1:4, C 1:4. To 2 c.c. of the diluted filtrate in a Folin Wu sugar tube were added 2 c.c. of the alkaline copper reagent, the mixture shaken, heated in boiling water for five minutes, cooled under running water, 2 c.c. of the complex tungstic acid color reagent added, allowed to stand two to three minutes, diluted to the 25 c.c. mark, shaken, and read in the colorimeter against the standard (0.2 mg. to 2 c.c.) glucose solution set at 30 mm.

Calculation—Mg. (carbs glucose) per c.c. medium =  $\frac{0.2 \times 30}{\text{Reading in mm}} \times \frac{1}{2} \times \text{Dilution} \times$

$$\frac{10}{4} = \frac{15}{2} \times \frac{\text{Dilution}}{\text{Reading}}$$

#### EXPERIMENTAL RESULTS

In every experiment with *B. coli* in the different carbohydrate media, the medium without the culture was analyzed as well as after incubation with the culture. Because of the method of preparing the media, especially the high concentrations of the carbohydrate solutions added, there was a certain variation in the chemical compositions of the individual media, although these differences were of small significance in the study of the utilization of the constituents by the *B. coli*.

The analyses of the media are given in Table I. The individual determinations are not included. Only the extreme values, the averages, and the number of determinations are given.

The pH determinations ranged from 6.8 to 7.3, values which are within the limits of accuracy used in the preparation and testing of the media. The averages of the titrable acidities of the different carbohydrate media were practically the same, 0.28 to 0.31, although individual values ranged between

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The determinations were carried out as follows:

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*pH*.—Indicators and standard controls were used.

addition of organisms) found in that experiment. Fermentable material was absent in all cases after the growth of the organisms so that these results are omitted from Table II.

## DISCUSSION

The first fact which appears in these results is the possible variation in any one individual experiment. It is necessary, therefore, to draw conclusions only from a series of experiments and to place limited weight on any one result. Most stress will consequently be placed on the averages.

TABLE II  
SOME CHANGES IN MEDIA DUE TO GROWTH OF ORGANISMS

MEDIA	NO OF DET'NS	DECREASES IN PH		TITRATABLE ACIDITY CHANGES CC 0.1 N NaOH		GAS PRODUCED MM		TOTAL REDUC- TIONS DECREASES CALC AS MG GLUCOSE PER CC	
		RANGE	AVERAGE	RANGE	AVERAGE	RANGE	AVERAGE	RANGE	AVERAGE
		(After feeding cane sugar as additional carbohydrate)							
Glucose	11	2.432	2.81	0.710	0.84	20.41	2.77	1.05222	1.53
Maltose	11	1.825	2.36	0.108	0.40	0.724	1.28	0.23104	0.68
Lactose	5	1.926	2.36	0.107	0.42	16.25	2.28	0.28094	0.53
A	6	1.925	2.34	0.104	0.23	12.23	1.72	0.45082	0.65
B	12	1.525	1.85	0.004	0.16	0.511	0.66	0.27072	0.49
C	11	1.826	2.20	0.106	0.33	0.319	1.21	0.22063	0.40
(After feeding carbohydrate C as additional carbohydrate)									
Glucose	6	2.632	2.77	0.609	0.73	20.32	2.50	1.34256	1.90
Maltose	3	0.103	0.20	0.0	0.0	0.0	0.0	0.12018	0.10
Lactose	3	2.528	2.70	0.406	0.53	1.21	1.80	0.39091	0.58
A	3	2.124	2.27	0.2	0.20	16.19	1.77	0.57077	0.66
B	6	0.517	1.03	-0.201	0.02	0.511	0.82	0.30058	0.40
C	5	-0.306	0.20	0.0	0.0	0.003	0.26	0.00020	0.10

The first half of Table II, where the infants had been fed their normal formula with cane sugar as the only added carbohydrate, the pH changes were largest for glucose media, smallest for carbohydrate B media, with the rest in between and much the same. Similar relations were shown for the titratable acidities and gas production. The changes in total reduction were largest for the glucose media, while the rest did not differ much from each other. A comparison of the reduction changes with the compositions of the media given in Table I shows that (a) for the glucose media, about 30 per cent of the glucose was destroyed, the growth of the organisms probably being stopped by the degree of acidity developed, (b) for the maltose media, about 18 per cent of the maltose was destroyed, no glucose being present before or after the culturing, (c) for the lactose media, about the same percentage of the lactose was destroyed but with apparently greater gas production, although the results are not sufficient in number to be entirely convincing, (d) for the carbohydrate A media, all the glucose present originally was destroyed but no other reducing substance attacked, (e) for the carbohydrate B media, all the glucose present originally was destroyed as well as a small amount of other reducing substance, possibly maltose, and (f) for the carbohydrate C media on the average approximately 30 per cent of the reducing substance, possibly maltose, was destroyed.

The results in the second half of the table where the organisms obtained after C feeding were cultured are average values. As stated before, they refer to maltose negative organisms, the dominant strain of the intestinal flora of one infant and specially selected strains of the intestinal flora of the other two infants. The results for glucose, lactose, and carbohydrate A media were practically the same as in the first half of the table; with carbohydrate B media, there was less breakdown as shown by the pH and reducing action changes, although the gas production was much the same. For maltose and carbohydrate C media there were practically no changes in the tests due to the culture, although the organisms had grown well as shown by the densities. This is in line with what would be expected of maltose negative organisms, while the changes in the carbohydrate B media were evidently due to the glucose present.

To sum up the results presented, so far as the compositions of the carbohydrate preparations used are concerned, C contained no glucose, B contained some, while A contained considerable. In the growing of the cultures in media containing the various carbohydrates, the glucose was used first. If there was no glucose present, maltose (with the maltose positive organisms) and lactose could be utilized to a certain extent. Also, with a small amount of glucose (as in B), after this was used up, maltose was attacked to some extent. The changes in pH, in titrable acidity, in gas production, and in total reductions, in general, ran parallel. The maltose negative strains tested utilized glucose, lactose, and A in the same way as the maltose positive strains, B to a slightly less extent (evidently only the glucose was attacked), while maltose and C (containing no glucose) were not utilized at all.

It is assumed in this discussion that in the breakdown of the starches in forming carbohydrates B and C, maltose and glucose are the only reducing substances formed. It is, of course, possible that other reducing substances, between dextrin and maltose in complexity, are present. The assumption was made for purposes of simplicity in the presentation of the problem and the discussion of the results. The presence of other reducing substances as indicated would change some of the quantitative deductions but not the general relations.

In media with the simple mono- and di-saccharides which were used here the change in pH, the acidity, and the gas formation were as a rule higher than in the mixtures containing carbohydrates A, B or C which are commonly used in infant feeding. An exception to this was carbohydrate A which contains saccharose and more glucose than carbohydrate mixtures B and C, the latter two consisting mainly of maltose and dextrin. Carbohydrate A showed more gas production than maltose. The maltose nonfermenting coli (twenty-four hours' incubation) showed neither acid nor gas production in cultures with carbohydrate C. If the idea presented in the introduction is correct the results suggest the conclusion that carbohydrate mixtures containing no glucose or carbohydrates which readily form glucose might be less apt to cause fermentation in the intestines than those which contain free or readily freed glucose.

Thanks are due Miss Margaret D. Schaffner for carrying out the determinations of the reducing action and acidity.

## SUMMARY

The growth of *B. coli* from intestinal flora of infants on media containing different carbohydrates was followed by means of changes in pH, total acid and total and fermentable reducing actions, and the gas evolved. The carbohydrates used included glucose, maltose, lactose, and three commercial preparations, i.e., a syrup, and two mixtures obtained by the partial hydrolysis of starch.

*B. coli* isolated from infant stools produces somewhat more gas from glucose than from lactose, considerably more gas from these two carbohydrates than from maltose. Of three commercial carbohydrate mixtures the one containing glucose and sucrose showed more gas production than those consisting simply of maltose and dextrin. Acid production showed differences of a similar order but to a very much less extent. Some of the *B. coli* strains did not attack maltose in twenty-four hours and thus showed still greater differences of the same nature.

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## A FURTHER CONTRIBUTION TO THE LIVER-KIDNEY SYNDROME\*

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IN PREVIOUS publications,<sup>1</sup> we have called attention to an apparently characteristic train of clinical symptoms and pathologic changes which began with hepatic damage and were followed by serious parenchymatous kidney degeneration. We have called this clinical and pathologic relationship between the liver and the kidney a "liver-kidney syndrome." Clinical and pathologic experimental study of this syndrome seemed to justify the assumption that its pathogenesis consisted essentially of a primary parenchymatous hepatic damage. This damage appears to be independent of both infection and ordinary cellular autolysis, and is followed by an elaboration of certain undetermined hepatogenic toxins or metabolites which act specifically upon the kidney tubules and by that action produce a severe and often fatal clinical picture terminating in many instances in a condition resembling uremia.

It has been suggested by Bartlett<sup>2</sup> in reviewing our cases that the kidney findings are those of acute nephritis due to infection in the liver. The question as to whether bacterial toxins or metabolites toxic to kidney parenchyma may be the important factors producing the kidney damage in the liver-kidney syndrome is, of course, highly speculative. In all of our cases, particularly those associated with gallbladder infection, we have attempted to eliminate infection as a factor by pre- and postmortem bacteriologic studies. We have observed the syndrome not only in cases of uncomplicated hepatic trauma, but also in metastatic carcinoma of the liver. In neither of these could cellular or cultural evidence of infection be demonstrated.

It has also been suggested that the etiologic factor in the production of the kidney changes in our cases has been a specific toxin produced by the liberation of autolytic products of asptic tissue necrosis similar to that seen in extensive burns and in pancreatitis with fat necrosis. In answer to this, it should be mentioned that aside from the hemorrhagic diathesis, so frequently an important clinical feature in the syndrome, no other parenchymatous organs show the changes at necropsy suggestive of such a process. In the toxicities of autolytic tissue necrosis, submucons hemorrhages are extremely rare. Moreover, the characteristic chain of clinical manifestations with the gradually increasing oliguria, low grade edema, uremia and the progressive increase in blood nitrogen are certainly not usual findings in this type of toxicity.

*Classification of the Renal Lesion.*—Although there is considerable difference of opinion regarding the classification of the nonsuppurative types of

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nephropathies, we have considered the kidney lesion in our liver kidney syndrome to be essentially a nephrosis. We base our classification upon the fact that the anatomic changes are chiefly degenerative rather than inflammatory. Whatever the cause may be, the result is a degenerative and not an inflammatory lesion of the kidney. There is in fact, evidence other than our own that such kidney changes may occur with or without the presence of bacteria. Thus, hepatonephrosis is almost constantly present in yellow fever where, not infrequently, the renal changes are in many ways similar to those found in our cases. On the other hand an almost identical kidney tubular lesion is found in certain cases of chemical poisoning. In both instances, the assumption seems justified that the degenerative kidney change is not a result of direct infection but rather that of a powerful toxin. Certainly in the renal lesions of the cases we have studied, there is no evidence of glomerular nephritis, the lesions are almost wholly confined to the tubular epithelium and the interstitial tissues are apparently undergoing degenerative changes.

We have attempted to demonstrate glomerular alterations by Mallory's aniline blue and Masson's polychrome stains which stains failed to reveal any of the characteristic anatomic alterations in the glomeruli characteristic of any stage of glomerular nephritis. Although some evidence of endothelial swelling was seen at times and in some specimens prominence of the epithelium was apparent control specimens taken from the kidney of a perfectly normal young woman, who died ten minutes after being struck in the jugular vein by a jagged piece of automobile windshield glass showed the same degree of these glomerular changes. Moreover these stains did not show any of the characteristic hyaline bundles of glomerular nephritis in the glomeruli of any of our cases and pus cells were never numerous while red blood cells were always present, at times abundantly so.

Although focal interstitial infiltrates consisting almost exclusively of mononuclear cells were often present it is our opinion that these represented a reaction to degeneration. The supposition that the presence of red blood cells in the urine indicated an acute glomerular nephritis can be controverted by the fact that in all instances in which red blood cells were found in the urine we were able to demonstrate submucous petechiae in the kidney pelvis and bladder, and we also always found microscopic focal hemorrhages in the medullary portion of the kidneys.

The pathologic picture of the kidneys in our cases bore no similarity to that of so called lipid nephrosis. We were able to reach this conclusion, since in no instance did either differential fat stains or polarized light reveal evidence of fatty degeneration or infiltration.

The changes found in the kidneys of the cases under discussion were in many important details very similar to the nephrosis found in certain cases of chemical poisoning in pregnancy toxemias and in other true advanced toxic nephroses.

In summarizing the histologic picture of the changes characteristic of the nephrosis in the liver kidney syndrome it is to be noted that the principal



finding is degeneration of the tubular epithelium in the more highly differentiated tubules, the degeneration ranging from a minor grade of cloudy swelling through vacuolar degeneration to actual epithelial necrosis. Although, as mentioned above, minor evidence of glomerular capillary and epithelial irritation was not infrequently seen, this was always of minor importance.

Another striking feature was the focal medullary cellular infiltration. Interstitial hemorrhages were very frequent and in many instances pigment-laden phagocytes filled with hemosiderin were found in these focal areas. Moreover, the cellular components of these infiltrates were almost exclusively round cells, eosinophiles and plasma cells, and every feature of the reaction suggested a reaction to degeneration.

#### CASE REPORTS

As a further contribution to the liver-kidney syndrome which we have described, we wish to present two cases, one of pure trauma where sections were taken from the liver in areas both adjacent to and within the pulpified region, and also throughout various parts of the nontraumatized liver. In none of these was there the least evidence of any cellular reaction which could be interpreted as the result of bacterial invasion. Our other case is one of pure liver infection in which multiple suppurating thrombi were found in the portal venous branches. In this case the clinical picture was that of progressive anuria and uremia with extensive blood nitrogen retention and almost exsanguinating mucous surface bleeding. At necropsy, the kidney showed the anatomic findings identical with those seen in the traumatic case where no evidence of any infection could be demonstrated.

**CASE 1.—History:** Shortly before admission to the hospital, the patient, a well-developed man of sixty-one years of age, was in an automobile accident. He walked into the hospital complaining of pain in his right chest and his right knee. Physical examination demonstrated a fracture of the right patella and the first to tenth ribs of the right anterior thorax and second and third ribs of the left thorax. His temperature was 99° F., his pulse 90. He had, previous to this accident, enjoyed excellent health all of his life.

Approximately twelve hours after his admission to the hospital, his temperature rose to 100° F., his pulse to 160, while his respiration remained normal. Complete laboratory examinations including urine, blood count, and blood chemistry were normal. His general condition remained about the same except for a moderate abdominal distention until the next day (the second day of his illness) when his abdominal distention increased, his temperature rose to 101° F., and he began to vomit small quantities of blood and pass large quantities of blood by rectum. On physical examination, tenderness was present over the entire right upper quadrant of the abdomen. He rapidly became toxic with occasional periods of muttering delirium. Despite the intravenous and subcutaneous injection of considerable quantities of glucose and saline, his urinary output rapidly decreased. Slight edema of the ankles and back appeared. Examination of the urine revealed the presence of a faint trace of albumin, hyaline casts and microscopic blood.

The next day (the third day of his illness) he became delirious, abdominal distention increased, and he vomited considerable quantities of blood. His bowels were incontinent and consisted almost entirely of blood. His temperature rose to 102° F., and his pulse remained at 160. His urinary output decreased to almost complete anuria but examination of small quantities revealed a heavy trace of albumin, 30 to 40 pus cells per high power

field of centrifuged urine, hyaline and granular casts, and microscopic blood. His blood count was normal but his blood chemistry showed a nonprotein nitrogen of 75 mg and a creatinine of 3 mg per 100 cc. His blood sugar fell to 58.8 mg per 100 cc.

From muttering delirium he passed into a deep coma and died in a state clinically resembling uremia.

*Necropsy.* Surface inspection of the body revealed numerous scratches and bruises and there was a faint lemon tint to the skin. In the peritoneal cavity some clear brownish fluid was present. Over the dome of the liver, a large mass of clotted blood was found and three cracks were present in the capsule. The deepest rent was found immediately to the right of the falciform ligament and this extended almost entirely through the liver. Lateral to this fracture, the hepatic parenchyma was pulpified and of a deep brick red color. This zone of pulpification extended from 2 to 4 cm into the liver substance all along the line of fracture. There was likewise considerable extension of the hemorrhage into the perihepatic

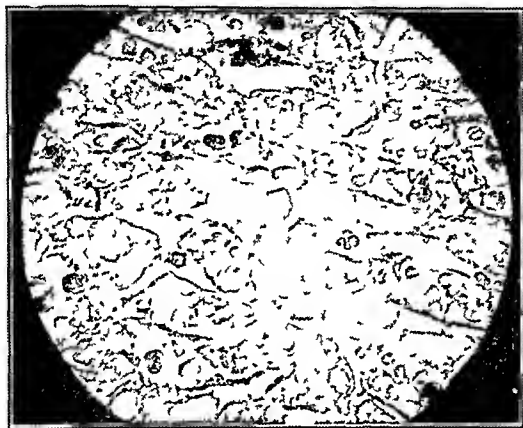


Fig. 1—Photomicrograph of the liver in Case 1 taken through border of necrotic zone. observe anuclear liver cells and lack of inflammatory reaction.

retroperitoneal tissue. The remaining liver parenchyma was not involved in the hemorrhage and showed a pale, swollen somewhat mottled surface. The liver weighed 1,400 gm.

The kidneys were somewhat swollen. The capsules stripped with ease and small pinpoint hemorrhages were seen in the medullary zones of both kidneys on cross section. The markings were very indistinct and the cut surfaces were pale and somewhat bulging. The ureters were negative. The bladder was contracted and there were about 5 cc of urine present which contained albumin, white cells, some granular casts and microscopic blood.

In the chest cavity nonpenetrating fractures of the first ten ribs of the anterior right thorax were found and the second and third ribs on the left side were cracked. Some hemorrhages were found under the epicardium of the left auricle and interstitial hemorrhages were present in both lungs.

There was considerable interstitial hemorrhage into the capsule of the right adrenal.

*Histologic Pathology.* Sections through the liver adjacent to the area of fracture and pulpification showed a zone of extensive hemorrhage in which masses of fibrin were buried. Here and there a few scattered necrobiotic leucocytes were found. The adjacent liver tissue showed varying stages of necrosis ranging from complete epithelial disintegration through

hyaline anuclear necrosis (Fig. 1) to varying degrees of parenchymatous degeneration. At the outer border of the zone of trauma and extending a considerable distance into the grossly unaltered hepatic parenchyma, focal interstitial hemorrhages were present, and the liver cells were quite intact except in these small focal areas. In the zone of pulpification, wide expanses of liver tissue were observed where the hepatic cells possessed no nuclei but still retained their outlines. There the sinusoids were intact and in most instances plugged with masses of red blood cells. There was no evidence of any inflammatory reaction, such as would be produced by an infection, in any of the sections viewed.

The kidneys showed different stages of cloudy swelling, vacuolar degeneration and in the more highly differentiated tubules there was even loss of nuclei in many cells. Many of the tubules contained masses of stringy albuminous material or casts. The glomeruli were perhaps slightly more cellular than normal, and between the tuft and the glomerular capsule occasionally thin stringy strands of albuminous precipitate were seen, but for the most part the glomeruli were relatively normal in appearance (Fig. 2). In the medullary portion of the kidney, small focal areas of hemorrhage were encountered as well as areas wherein large



Fig. 2.—Photomicrograph of the kidney in Case 1; note very minor degree of glomerular change (Mallory's aniline blue stain).

numbers of phagocytes heavy laden with pigment were seen. These large pigment cells were bulging with deep brown hemosiderin granules. Occasional scattered round cells were likewise seen in these foci. Many of the tubules in the medullary portion likewise contained albumin or casts. Special stains for the glomerulus showed only minimal changes.

The mucosa and submucosa of the stomach showed extensive hemorrhage and some edema. Scattered polymorphonuclear leucocytes and round cells were present in the stroma.

The other organs revealed nothing particularly noteworthy histologically.

**CASE 2.—History:** The patient was a well-developed farmer forty-two years of age. He had been perfectly well until four days before his admission to the hospital. Although he had not seen a doctor until just before his admission, he gave a history of symptoms typical of appendicitis. His physical findings were those of a perforated appendix with local peritonitis. His temperature was 100° F., his pulse 100, and his respiration 30. His blood picture was normal except for an increase in leucocytes to 11,000. His urine was highly concentrated and contained a trace of albumin; otherwise, it showed no abnormalities. The

following day, the fifth day of his illness, he became lightly jaundiced and passed a considerable amount of dark blood by rectum. The next morning his jaundice had deepened and he began to bleed from his mouth and lips. He also coughed up blood stained sputum and he continued to pass blood by rectum. At this time his urinary output decreased rapidly to almost complete anuria. The urine contained albumin 1 plus, 20 to 30 red blood cells to the high power field and an occasional pus cell. His blood count showed 4,320,000 red cells, 8,250 leucocytes with 80 per cent polymorphonuclears and 20 per cent small lymphocytes. Blood chemistry determinations revealed a nonprotein nitrogen of 1.0 mg creatinine 3.7 mg, chlorides 330 mg, and blood sugar 118 mg per 100 cc of blood. His temperature rose to 101° F, pulse to 110, and respiration to 35. During the next twenty four hours, the seventh day of his illness the bleeding continued from his mouth, lips, lungs, and intestinal tract. The jaundice remained practically unchanged. The urinary output further decreased until it was difficult to get a sufficient sample for examination. The urine contained a trace of albumin, 20 to 30 red cells to the high power field, granular casts, a small amount of bile, and an occasional pus cell in the uncentrifuged specimen. The blood nonprotein nitrogen rose to 285 mg and the creatinine to 4.5 mg. The blood sugar fell to 51.2 mg and the blood chloride increased to 430 mg per 100 cc of blood. The blood count at this time showed 2,150,000 red cells and 11,800 leucocytes with 72 per cent polymorphonuclears and 28 per cent lymphocytes.

Toward the latter part of the seventh day of his illness, the patient died with symptoms suggesting uremia.

**Necropsy** Both clotted and unclotted blood were found in the mouth and nose. The skin was jaundiced. The abdomen was distended. When the abdominal cavity was opened the gutter along the cecum and ascending colon was filled with flocculent semipurulent, bloody exudate and the cecum and ascending colon were distended, their walls greatly thickened and indurated and of a deep bluish black color. The mucosa was extensively ulcerated and a gangrenous appendix was found lying in a walled off pool of foul smelling exudate.

The liver was large and hard and on section pale and shining. When pressure was applied to it, focal pinpoint to pinhead size dots of milky pus appeared in clusters on the cut surface. The superior mesenteric and portal veins were filled with semilytic suppurating thrombi. The stomach and entire bowel contained a large amount of bloody feces. The kidneys were greatly enlarged, their capsules tense, and on section the renal parenchyma bulged out under the incised capsule. The markings between the cortex and medulla were very indistinct. Both kidneys were definitely jaundiced. Numerous petechial hemorrhages were visible in the pelvis. Both lungs showed subpleural and interstitial hemorrhages and edema and terminal bronchopneumonia was present in each lung.

**Histologic Pathology** The liver showed a rather diffuse, low grade, parenchymatous degeneration. There was considerable swelling and even desquamation of many of the Kupffer cells and scattered here and there throughout the sinusoids leucocytes were found. In the portal areas a diffuse infiltration with round cells and scattered polymorphonuclear leucocytes was seen. In some of the very smallest portal venous channels, masses of suppurative thrombi were seen (Fig 1). Moreover in the larger portal ramifications, purulent necrotic exudate was found and in some areas there was considerable fragmentation of the vein walls. However, very little extension of the exudate had taken place into the liver proper. Under the higher magnification there was no evidence of obstructive jaundice except occasionally near the center of the lobule where the parenchymatous cells contained scattered deposits of brownish pigment and in some of the small bile canaliculi minute bile thrombi were present. The liver cells in many instances had quite indistinct outlines and showed varying degrees of nuclear lysis. Many of the Kupffer cells in addition to being tremendously swollen and desquamating, contained some pink semigranular material of undetermined character. There were definite hemorrhages in the gallbladder mucosa.

The kidneys showed striking tubular changes the most interesting of which was an extensive degeneration. This change varied from low grade cloudy swelling to frank epithelial necrosis. In some sections focal leucocyte infiltrations, in which either round cells or plasma

cells predominated, were scattered in the medulla and cortex. In addition to these cells, pigment containing phagocytes and other evidences of previous interstitial hemorrhage were apparent. Fresh interstitial hemorrhages were also noted in the medullary interstitial tissue.

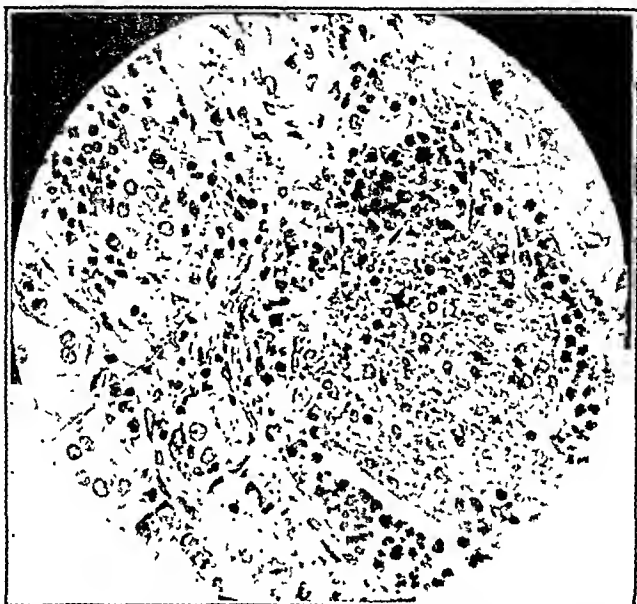


Fig. 3. Photomicrograph of the liver in Case 2, showing a thrombus in a small portal radicle and interstitial pus cell infiltration.



Fig. 4.—Photomicrograph of kidney glomerulus in Case 2; glomerular changes are negligible (Mallory's aniline blue stain).

Many of the tubules where the necrosis was not so marked showed a generous accumulation of albuminous precipitate in their lumina. In other areas the outlines of the tubular epithelium were so irregular and indefinite that one gained the impression that most of the

cells were beginning to slough away. In many tubuli, hyaline droplets were present in enormous numbers. Special stains were employed for glomerular epithelium and basement membrane, however. With these the glomeruli showed but minor changes consisting of a low grade swelling of the capsular epithelium and some proliferation of the endothelium (Fig. 4). Occasionally some of the tubular cells had a faint brownish tinge suggesting imbibition of bile, but this was by no means striking.

The lungs showed edema, early bronchopneumonia, and in the edematous fluid large numbers of red cells in varying stages of hemolysis could be seen.

The appendix showed the typical picture of acute suppurative appendicitis with suppurative thrombi in the venous channels and beginning gangrene of the cecal wall.

*Discussion of Cases*—We consider the following points in Case 1 important in the proper evaluation of the syndrome. First, there was no evidence of infection. Second, although the patient was faintly jaundiced toward the end of his illness, there was no microscopic evidence of bile pigment in the renal epithelium. It is also interesting that the area of fracture and hepatic pulpification was in the middle of the liver between the right and left lobes. This location of the pulpification was similar to that observed and previously reported by us in the liver of a patient who presented this same syndrome. It should be emphasized that Case 1, prior to the accident, had been a well and apparently normal individual and that the first clinical manifestation of renal impairment appeared on the third day after the injury. The kidney lesion was predominantly tubular in character, the changes being those of varying degrees of parenchymatous degeneration with but few minor evidences of glomerular damage. Although some old foci of glomerular fibrosis were present, these changes were not inconsistent with the common findings in the kidneys of persons of this same age dying from some totally unrelated disease. This latter finding, therefore, was considered unimportant. Clinically the patient vomited blood and passed blood per rectum and at necropsy submucous hemorrhages were present in the gastrointestinal tract. This gastrointestinal bleeding is very frequent in these cases and when present, is one of the outstanding features of the syndrome. Such bleeding is prominent not only in the traumatic cases, but has also been observed in the great majority of cases in which the fatal syndrome has followed other forms of liver damage.

In sharp contrast to Case 1 two factors were present in Case 2 which might be considered as of possible influence in the production of the marked renal lesion found at necropsy, i.e., infection and jaundice. We have included Case 2 because we wish to show that the renal picture produced by pure hepatic trauma unassisted by either jaundice or infection, may produce the same clinical and pathologic picture as that observed in cases presenting the liver-kidney syndrome which are not associated with infection.

In Case 2 the liver abscesses were, for the most part, of microscopic dimensions, in only a few areas could they be recognized with the naked eye. When pressure was applied to the liver, minute droplets of pus oozed out on the cut surface. Histologically these were found to be small portal vein thrombi surrounded by areas of advanced parenchymatous degeneration proceeding in many regions nearly to necrosis of the hepatic cells. The patient in Case 2 was considerably jaundiced and grossly and microscopically the

kidneys showed some staining with bile. With the exception of this addition to the gross and microscopic picture of the kidney, the renal changes were in all respects comparable to those found in the nonjaundiced, noninfectious case of traumatic liver pulpification. Moreover, clinically, aside from the symptoms of acute appendicitis and ascending pyelophlebitis with jaundice, the sequence of clinical manifestations such as gradually increasing oliguria proceeding to anuria, rapidly mounting blood nitrogen and extensive bleeding from the mouth, stomach and bowel, duplicated in all respects those findings observed in the traumatic case.

The renal lesion found in these cases was very interesting. The glomeruli showed but minor alterations; in some instances slight accumulations of albumin had occurred in the capsular space, while in others there was a slight epithelial proliferation and endothelial swelling as well as a low grade thickening of the glomerular membrane. These changes, however, were minimal and negligible when compared to the remarkable degenerative changes found in the tubules.

The classical picture of bile nephrosis so frequently encountered in obstructive jaundice was duplicated in the renal picture in certain phases only. Fahr<sup>3</sup> mentions the presence of fatty changes in the renal epithelium in addition to the universal or streaked distribution of bile pigment in the tubules, Bowman's capsule, and intratubular albumin. In only one case in his experience was there evidence of localized inflammatory lesions. Our Case 2 showed bile only in minor quantities and the impression was gained from careful microscopic study that something aside from the infiltration of bile was responsible for the overwhelming tubular damage.

The presence of occasional small foci of round cells and plasma cells in the medullary portion of the kidney was considered by us to be evidence of a reaction to a degeneration because granular debris was present in these foci although no pus cells were found. The presence of large numbers of cells bulging with hemosiderin pigment indicated that these cellular elements represented a reaction to a degenerative process which had doubtless been accompanied by some degree of hemorrhage. This latter hypothesis was substantiated by the finding of other fresher interstitial hemorrhages into which no leucocytes had as yet wandered.

*Literature.*—In a previous report, we recorded several cases, similar to those presented here, which had already been reported in the literature but in which no attempt had been made to explain the apparent relationship between the liver and the kidney.

McKnight<sup>4</sup> reported the case of a healthy boy nineteen years old who suffered a rupture of the superior surface of the right lobe of the liver as a result of an automobile accident, and at operation the rent in the liver was packed in the usual manner. Postoperative convalescence was uneventful until the sixth day when the patient became nauseated, jaundiced, and developed generalized abdominal distention. Laboratory findings at this time demonstrated a blood urea of between 240 and 270 mg. per 100 c.c. of blood. Albumin and granular and hyaline casts appeared in the urine. The leucocyte

count during this time ranged as high as 72,000. After three weeks of symptoms highly suggestive of uremia, the patient recovered but on dismissal still showed a trace of albumin and a rare cast in the urine. This history suggests a parallel to the histories in our syndrome cases.

Also, in 1931 Wulsten<sup>7</sup> observed an eleven-year-old schoolgirl who suffered a trauma to the liver while riding on a sled. Some time following the injury she bled from the nose and vomited blood. She became progressively anemic and red cells appeared in the urine. The red cell count and hemoglobin continued to decline until the hemoglobin was 19 per cent and the red count 1,500,000. After a rather long illness the patient died. At necropsy, although the capsule of the liver was intact, a large cavity was present which involved almost the entire right hepatic lobe and which was filled with dirty brown fluid. Multiple subperitoneal and subpericardial ecchymoses were present and many punctate hemorrhages were seen in the mucosa of the bowel and in the kidney pelvis. No histologic record of the kidney was given. In the liver, there was no microscopic evidence of infection but necrotic foci were found in the liver tissue surrounding the cavity. It was Wulsten's opinion that the mucous surface bleeding may have been due to absorption of toxic products from the necrotic liver tissue. Unfortunately no blood chemical studies were mentioned.

These two cases are presented only as additional circumstantial evidence. Further research of the literature of the liver and kidney shows that, although cases of this general character have not frequently been recorded, they may not be rare. This is brought out in a report by Stanton<sup>8</sup> who analyzed the cause of 500 deaths following gallbladder operations. In this series, he noted that twenty-four died of renal failure, chiefly anuria.

In 1927, Wilensky and Colp<sup>7</sup> published their observations regarding the retention of nitrogen bodies in cases of biliary tract disease. They noted that in ordinarily mild or moderately severe cases disturbances in nitrogen metabolism were not observed prior to operation. They also mentioned that such patients were frequently not operated upon until the essential lesion was relatively far advanced, so that nephritis (renal epithelium degeneration) was frequently present at the time of observation.

Since we called attention to this syndrome as a definite clinical and pathologic entity, other observers have reported cases of similar character. Thus Cole<sup>8</sup> of Washington University, in a recent discussion of hepatic insufficiency, cites the case of a woman forty-eight years old who from a clinical standpoint, appeared to be a good operative risk. Cholecystogastrostomy was performed for common duct obstruction. A few days after the operation she developed edema of the ankles and albumin and large numbers of granular casts appeared in the urine. There was a progressive oliguria which finally terminated in total anuria. The nonprotein nitrogen rose to 60 mg per 100 c.c. of blood, she was drowsy and irrational, and died on the ninth postoperative day. At necropsy, the liver showed extensive cellular necrosis and atrophy while the kidneys presented only moderate parenchymatous changes.



Hemorrhages were present in the walls of the renal pelves, and some parenchymatous hemorrhages were likewise seen.

It is Cole's opinion that infection played little part in the hepatic insufficiency and he likewise stated that he considered that an associated kidney damage in cases of severe hepatic disease to be extremely common and important.

Even more recently Eiss,<sup>9</sup> in a discussion of conservation of hepatic function in gallbladder operations, states that in his experience in biliary tract surgery he has observed "several cases of postoperative death without infection or hemorrhage with rapidly rising fever, rapid pulse and the terminal uremic syndrome." He is convinced that these cases are much more frequent than the number of reported cases would indicate and "that the tragedy of these deaths is that they occur in patients who appear clinically to be good operative risks."

Similarly, Heyd<sup>10</sup> in an editorial in *Surgery, Gynecology and Obstetrics* describes a type of "liver-death" as follows:

Previous to operation these patients have had what was considered normal kidney function and no question was in the mind of the surgeon as to the competence of the kidney to carry on its function in the presence of an operative intervention. Forty-eight hours after an operation on the gallbladder or common duct, the patient quite rapidly presents the picture not dissimilar to shock, with cold, clammy skin, gradual failure in water elimination and a rise in urea nitrogen. The urinary output becomes less and less, and a mild delirium develops with increased frequency of pulse and temperature, and finally coma and death. These patients are not jaundiced either before or after operation, and there is a distinct interval of apparently normal postoperative conduct of from twenty-one to thirty-six hours between the operation and the onset of the terminal clinical picture.

Previous to the publication of our first article,<sup>1</sup> Heyd<sup>11</sup> had considered this type of "liver-death" as a condition of hepatic exhaustion related in some way to the pancreas.

*Theories of Toxin Pathogenesis.*—Our cases in which the liver-kidney syndrome appeared following traumatic fracture of the liver now are two in number; one was reported in an earlier paper. In both instances, as mentioned above, the traumatic lesion was situated in the right lobe near the midline and was so placed as most easily to cause trauma of the main intrahepatic branches of the hepatic artery.

That extensive hepatic necrosis, even at the site we have discussed, may likewise fail to cause fatal kidney changes can be illustrated by the case reported by McKnight.<sup>4</sup> There seems little doubt that his patient recovered from a definite liver-kidney syndrome, but it is significant that three weeks later the patient continued to show albumin and casts in his urine, showing that very definite kidney damage had been effected.

Recently we have been fortunate in that Dr. T. G. Orr has permitted us to observe two cases of traumatic liver necrosis which were subjected to laparotomy and in which the clinical and blood chemical findings showed all of the features of the liver-kidney syndrome except the final anuria. These two patients recovered and now have normal kidney functions; these cases will be reported by Dr. Orr.

We have also seen several cases in which, during an acute exacerbation of chronic cholecystitis, albumin, red cells and casts appeared in the urine and a rise occurred in the nonprotein blood nitrogen, in one instance to 65 mg per 100 cc of blood. These patients have recovered with apparently normal kidney function. Incidentally, we have likewise observed cases which, following cholecystectomy, showed minor evidences of kidney damage and have subsequently recovered.

In this syndrome many puzzling and apparently contradictory points are brought to mind. From our clinical and experimental observations, it does not appear possible that the toxin is the product of pure hepatic necrosis since, in almost all of the cases in which the liver kidney syndrome appeared, hepatic necrosis except in the traumatic cases was not a striking finding. Moreover, in experimental ligation of the hepatic artery as well as in experimental hepatic trauma with packing of the central portion of the liver, we have found that the most extreme degrees of hepatic necrosis were, in many instances not followed by nitrogen retention or kidney damage, while in occasional instances definite tubular degeneration and nitrogen retention were observed although liver necrosis of a very much less degree had occurred.

In the human being, ligation of the hepatic artery has been performed in a number of instances. Recently Shann and Fradkin<sup>17</sup> recorded such a case in which massive infarction took place in the right lobe. This infarct, measuring 10 by 8 by 5 cm, was removed at a later operation. No mention of any renal complication was recorded. Several cases of traumatic liver necrosis with massive sequestration are mentioned in the literature. For example, Biernath and Feitig<sup>18</sup> record a case in which the entire left lobe of the liver became necrotic and was surgically removed. In this case likewise, no kidney complication was mentioned.

Many important things are known regarding the rôle of the liver in protein metabolism and the remarkable detoxifying powers of the liver against both endogenous and exogenous poisons have been demonstrated many times. Thus Rolleston and McNee<sup>14</sup> mention the frequent association of a severe renal insufficiency with retention of nitrogen bodies in the blood in cases of severe hepatic disease.

It is interesting in this regard that in certain cases of ilec fistula, when part of the portal circulation had been shunted into the general circulation and high protein diets were instituted, severe and even fatal toxemias have occurred. Moreover, it has also been demonstrated that a high protein diet has resulted in severe or even fatal toxemia in dogs that have had large portions of their portal blood shunted back into the general circulation as the result of the liver cirrhosis produced by carbon tetrachloride.

These observations seem to suggest the idea that the pathogenesis of the liver kidney syndrome depends primarily more upon the development of some specific type of intracellular hepatic damage than upon the degree of actual morphologic cellular damage to which ordinary cell injury may extend.

When we consider the wide variety of liver lesions and the more constant sequence of clinical events in which the renal picture assumes the most promi-

nence as the syndrome progresses, it appears more and more convincing that some toxin is the causative factor. This toxin may have been produced as the result of a perversion of function of damaged liver cells or by a lack of some physiologic detoxifying function of the liver parenchyma which permitted the production or the accumulation of a substance highly toxic to renal function.

The occurrence of hemorrhages in severe liver disease has been a frequent observation for a half century, but in most instances it has been attributed to a delayed coagulation time resulting from icterus. As far back as 1880, Schiff<sup>15</sup> noted that there occurred changes in the coagulation time which he felt were etiologic factors in the "hemorrhagic diathesis" associated with hepatic disease with and without icterus. The clotting time was found by Mann and Bollman<sup>16</sup> to be first decreased and then increased in total hepatectomy in dogs, and he observed marked oozing from all the abraded surfaces in those animals which had been kept alive for some hours. The clotting time may remain normal even though fibrinogen may be decreased although, as Whipple and Foster<sup>17</sup> have shown, the clots may be so delicate that they will not stop hemorrhage. Mucous surface hemorrhage has been one of the particularly puzzling complications of the liver-kidney syndrome and in many instances this has been one of the most striking features in the clinical picture. Whether this is due to hepatic damage or renal insufficiency resulting from the action of some toxin which directly affects the capillary endothelium, is a matter of conjecture. Rolleston and McNee<sup>18</sup> have observed general hemorrhages in the late stages of hepatic cirrhosis, and they believe that "when the liver has become incapable of stopping poisons absorbed from the alimentary canal," a state of general hepatic toxemia results with a low blood fibrinogen content with subsequent damage to the blood vessels. They have seen petechiae of the skin and bleeding from the mucous membranes of the mouth and gums. In one case recorded by Webber,<sup>19</sup> hemorrhage took place even in the external ears, in addition to hemoptysis and bleeding from the gums. Rolleston and McNee<sup>20</sup> likewise cite a case recorded by Lendet where hemorrhage took place from the external ear in cirrhosis. A hemorrhagic diathesis is also not a rare complication in certain cases of renal disease where no accompanying liver lesion is present. At present, however, the true etiology of such bleeding is not completely understood.

#### CONCLUSIONS

1. A brief description of the liver-kidney syndrome is outlined and questions of etiology are discussed.

2. An attempt is made to classify the renal lesion in this syndrome as a toxic nephrosis.

3. Two illustrative cases of the liver-kidney syndrome with complete necropsy studies are recorded and special microchemical studies of the kidneys are described. These two cases are recorded in an attempt to illustrate certain theories regarding the possible pathogenesis of the liver-kidney syndrome.

4. Additional cases are recorded from the literature which may be examples of the liver-kidney syndrome.

5 A theory is advanced to account for the elaboration of an hypothetical toxin believed to be responsible for the renal damage in the liver kidney syndrome, and mention is made of experiments in which uniform results could not be obtained when an attempt was made to reproduce the liver-kidney syndrome in animals

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## THE INTRANASAL APPLICATION OF INSULIN\*

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SINCE the discovery of insulin investigators have attempted to introduce it into the body by various methods. Woodyatt<sup>1</sup> in 1922 said "experiments were conducted with oral, rectal, vaginal, intranasal, intravenous, and subcutaneous administrations. Inunctions were also tried. Many variations were attempted in connection with each. Positive effects were obtained with subcutaneous and intravenous injections, very weak, doubtful or frankly negative results with the others." Up to the present time intravenous and subcutaneous administrations have been employed to the exclusion of all other methods.

Telfer<sup>2</sup> in 1923 reported that insulin could be introduced into the blood stream by means of inunction. Harrison<sup>3</sup> in 1926 repeated this work and found that the inunction of insulin was useless even in very large doses. Peskind, Rogoff and Stewart<sup>4</sup> in 1924 found that insulin when injected per rectum into rabbits was absorbed and produced lowering of the blood sugar; in dogs, negative results were obtained. Heubner, de Jongh and Laquer<sup>5</sup> in 1924 described the lowering of blood sugar in diabetic patients by inhalation of insulin. Fisher<sup>6</sup> in 1924 found some absorption of insulin by the intestine, vagina, and scrotal sac. Gänsslen<sup>7</sup> in 1925 described lowering of the blood sugar in diabetic patients by inhalation of an insulin spray. Miller<sup>8</sup> in 1926 reported that insulin given in absolute alcohol or 95 per cent alcohol solution, in keratinized capsules, lowered the blood sugar level of diabetic patients. Stephan<sup>9</sup> in 1929 described lowering of blood sugar following the administration of insulin by mouth. This work was not confirmed by Wahneau and Bertram<sup>10</sup> or by Bertram, Horwitz and Wahneau.<sup>11</sup> Bollman and Mann,<sup>12</sup> working with intestinal catheters and with ileac loops, found that large amounts of insulin might be instilled into the duodenum, jejunum, or ileum without any appreciable effect on the blood sugar of normal dogs. Similar results were found with administration into the ileac loop.

A summary of these results bears out the initial statement of Woodyatt that with methods other than subcutaneous or intravenous injection "very weak, doubtful or frankly negative results" have been obtained.

Recently we have repeated some of these experiments with variations, and, in the course of our work, studied the problem of intranasal absorption. We have obtained undoubted evidence of the activity of insulin, either when sprayed or when instilled into the nostrils in normal rabbits, normal dogs and in diabetic patients, under certain conditions.

Preliminary experiments indicated that the instillation or insufflation of insulin in the nose produced either frankly negative or doubtful results. We studied next various solutions which might possibly increase absorption

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TABLE I

DATE	ANIMAL	TIME	BLOOD SUGAR MG
3/15/35	Rabbit No 1	8 30 A M	247 0
		8 55 A M	02 cc insulin ethylene glycol mixture (100 units) intranasally
		10 28 A M	177 0
		11 47 A M	140 0
3/15/35	Rabbit No 2	9 05 A M	105 5
		9 08 A M	02 cc insulin ethylene glycol mixture (100 units) intranasally
		10 40 A M	62 5
		12 40 P M	55 2
3/20/35	Dog No 1	9 50 A M	96 0
		9 5 A M	04 cc insulin ethylene glycol mixture (200 units) intranasally
		10 50 A M	58 8
		11 45 A M	58 3
		12 45 P M	44 2

The effect of this solution when applied intranasally in diabetic patients is shown in the following

DATE	PATIENT	TIME	BLOOD SUGAR MG
3/18/35	No 1	7 30 A M	150
		8 15 A M	02 cc insulin ethylene glycol mixture (100 units) intranasally
		9 15 A M	149
		10 15 A M	122
		11 15 A M	83
		12 15 P M	110
3/19/35	No 2	7 30 A M	135
		8 15 A M	02 cc insulin ethylene glycol mixture (100 units) intranasally
		9 15 A M	87
		10 15 A M	94
		10 15 A M	02 cc insulin ethylene glycol mixture (100 units) intranasally
		11 15 A M	69
		11 15 A M	Slight reaction
		11 30 A M	More marked reaction
		11 40 A M	Orange juice
		12 15 P M	115
3/21/35	No 3	8 00 A M	225
		8 50 A M	02 cc insulin ethylene glycol mixture (100 units) intranasally (spray)
		9 50 A M	197
		10 50 A M	172
		11 50 A M	148
		12 00 NOON	02 cc insulin ethylene glycol mixture (100 units) intranasally (spray)
		1 00 P M	137
		2 00 P M	116
3/21/35	No 4	8 00 A M	256
		8 50 A M	02 cc insulin ethylene glycol mixture (100 units) intranasally (spray)
		9 50 A M	154
		10 50 A M	125
3/29/35	No 5	11 50 A M	115
		8 30 A M	188
		9 30 A M	180
		9 40 A M	02 cc insulin ethylene glycol mixture (100 units) intranasally
		10 30 A M	144
		11 30 A M	142

TABLE I—CONT'D.

DATE	PATIENT	TIME	BLOOD SUGAR MG.
3/30/35	No. 6	12:15 P.M.	158
		12:20 P.M.	0.2 c.c. insulin-ethylene glycol mixture (100 units) intranasally
		1:15 P.M.	106
		2:15 P.M.	65
		3:15 P.M.	66
4/ 3/35	No. 7	10:45 A.M.	286
		10:50 A.M.	0.2 c.c. insulin-ethylene glycol mixture (100 units) intranasally
		11:45 A.M.	112
		12:45 P.M.	67
		1:45 P.M.	73

through the mucous membrane. Finding several solutions which apparently had this effect, we chose ethylene glycol as a medium. We have employed solutions containing equal quantities of ethylene glycol and insulin, and have also made solutions of powdered insulin in ethylene glycol. The solutions employed for instillation contained 500 units per c.c. With such a solution 0.1 c.c. contains 50 units and 0.2 c.c. 100 units of insulin.

Table I shows a few typical protocols on animals.

#### CONCLUSIONS

Table I, which is an example of a larger group of similar observations, shows that insulin in ethylene glycol when either dropped or sprayed into the nasal mucous membrane produces an unquestioned and marked fall in blood sugar in normal rabbits, normal dogs, and in diabetic patients. The dosage employed by this intranasal method is considerably greater than that necessary in subcutaneous injection. The fact that insulin under certain conditions can be absorbed from mucous membranes is possibly of more than academic interest.

We are under obligations to Eli Lilly and Company for the insulin used in these observations.

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## FUNGICIDES\*

### I INFLUENCE OF HYDROGEN ION CONCENTRATION ON THE GROWTH OF YEASTLIKE ORGANISMS

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SINCE vulvovaginal mycoses often did not respond well to infrequent local applications of gentian violet and to sodium bicarbonate douches,<sup>6, 7</sup> it was deemed advisable to evaluate the therapeutic efficacy of other agents. Such a problem involved the investigation of numerous compounds, many of which were acid or alkaline or required an acid or alkaline vehicle for solubility or stability. The effects of various hydrogen ion concentrations on the growth of certain test fungi were studied so that this factor might be recognized in subsequent work with antiseptic solutions. Nine strains of pathogenic fungi representative of those isolated from human patients, and a known nonpathogen, *Saccharomyces cerevisiae*<sup>7</sup> were employed. The hydrogen ion concentrations varied from pH 2.5 to 9.5 since these limits were well beyond those present in physiologic or pathologic vaginal discharges<sup>1, 4, 8, 9, 10</sup> or therapeutically practicable.

#### TECHNIC

Two types of media were employed: (a) 5 per cent glucose solution and (b) 0.5 per cent glucose in broth (veal infusion—peptone), each containing sodium monobasic phosphate as a buffer. Although the sodium monobasic phosphate was recognized to be scarcely adequate for these extreme limits it was chosen so that any possible fungicidal effect would be constant throughout the study. The original medium was made up to pH 5.5, since fungi grew well at this level on dextrose peptone agar slants and in glucose broth. Several volumes of 75 cc of medium were adjusted to the various selected hydrogen ion concentrations by the addition of solutions of N/2 NaOH or N/5 HCl, after which 10 cc amounts were distributed into culture tubes for subsequent inoculation. By the colorimetric spot plating method the approximate values of pH 2.5, 3.5, 4.5, 5.5, 6.5, 7.5, 8.5, and 9.5 were determined,<sup>2</sup> and the medium was rechecked by the same method after the growth of the fungi. The color indicators were prepared according to standard routines<sup>3</sup> for thymol blue, bromphenol blue, methyl red, bromthymol blue, and cresol red, and were used as prescribed by Clark.<sup>2</sup> Even though this method indicated only approximate pH values it was sufficiently accurate for this study.

Ten strains of organisms representing three different genera were used. These were *saccharomyces*, *cryptococci* and *monilia*.<sup>3, 9</sup> In view of the con-

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fusion which existed in mycotic taxonomy, certain cultural characteristics are indicated in Table I. Strain F40 (one of the bakers' yeasts) was a proved nonpathogen,<sup>7</sup> while the remaining cultures were obtained from patients with vaginal or oral mycoses.

TABLE I\*

	DEXTROSE	LEVULOSE	MANNOSE	MALTOSE	GALACTOSE	SACCHAROSE	LACTOSE	MILK	MYCELIA	ASCOSPORES	CLASSIFICATION ACCORDING TO	
											MORPHOLOGY	FERMENTATION
Saccharomyces												
F40	⊕	⊕	⊕	⊕	⊕	⊕	-	-	-	P	Cerevisiae	Cerevisiae
149	⊕	⊕	⊕	⊕	⊕	⊕	-	-	-	P	?	?
Cryptococcus (Torula)												
119	⊕	⊕	⊕	-	-	-	-	-	-	-	?	?
Monilia												
											Benham	Stovall
104	⊕	⊕	⊕	⊕	⊕	+	-	C	P	-	Albicans	Type 2
120	⊕	+	⊕	⊕	+	+	-	C	P	-	Albicans	Type 2
147	⊕	⊕	⊕	⊕	⊕	-	-	-	P	-	?	Type 1
152	⊕	⊕	⊕	⊕	⊕	⊕	-	-	P	-	Candida	Type 3
155	⊕	⊕	⊕	⊕	⊕	⊕	-	-	C	P	?	Type ? (variable)
156	⊕	⊕	⊕	⊕	⊕	⊕	-	-	C	P	Albicans	Type 2
157	⊕	⊕	⊕	⊕	⊕	+	-	-	P	-	Parapsilosis	Type 1
*+ = acid    O = gas    - = negative    C = coagulation    P = produced    ? = undetermined												

Subcultures were grown on Sabouraud's agar slants and incubated at 37° C. for twenty-four hours. A small loopful of the growth was then transferred to sterile 0.9 per cent NaCl solution in sufficient quantities to make a suspension containing either 50,000 or 500,000 cells in 0.1 c.c. The actual cell counts were made with a standard hematimeter. One-tenth cubic centimeter of the suspension was transferred immediately to each of the 10 c.c. of medium, previously adjusted to the various pH levels, as shown in the tables, and incubated at 37° C. for from eighteen to twenty-four hours, after which one-fiftieth (0.02) cubic centimeters of the suspension of each of the thoroughly agitated cultures was transferred to a Petri dish by using finely drawn pipettes. About 15 c.c. of Sabouraud's medium were then poured into the Petri dish and the culture was mixed by gentle manipulation. Colony counts were made without the use of a magnifying lens following incubation at 37° C. for from twenty-four to one hundred and twenty hours.

## RESULTS

*Glucose Medium.*—To determine the rate of cell reproduction in 5 per cent glucose (Table II and Chart 1), an inoculation of approximately 2,000 yeast cells per 0.02 c.c. of medium was made in each of the original and duplicate tubes for Strains 119, 152, 155, and 156. From actual cell counts, as shown by the circles on the graph, it is evident that many yeast cells died at the low hydro-

gen ion level, while some died even at the supposed optimum level of pH 5.5. The horizontal line represents the number of cells transferred from the original suspension. In general, the rate of growth increased progressively as the acidity approached pH 5.5 from either extreme. In culture No. 152 duplicate, at pH 5.5, the decrease may have been the result of an incomplete suspension of cells before transplantation or insufficiently cooled Sabouraud's medium.

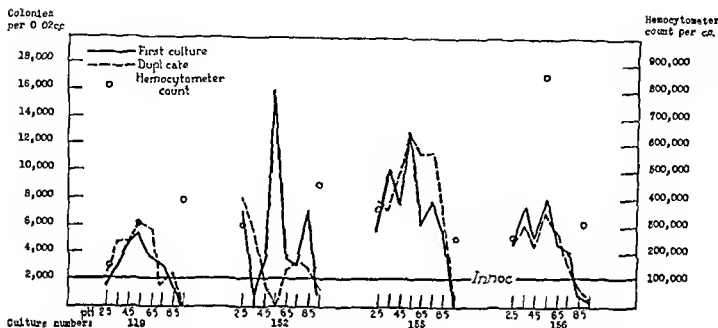


Chart 1—Hemocytometer and poured plate counts after forty eight hours incubation. Medium 5 per cent glucose.

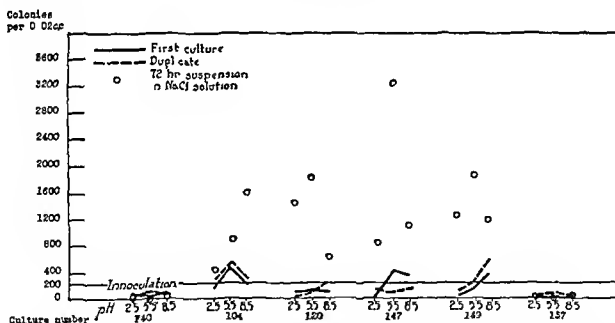


Chart 2—Poured plate counts from fresh and seventy two hours 0.90 per cent NaCl suspensions. Medium 5 per cent glucose.

The remaining six strains (F 40, 104, 120, 147, 149, and 157) were inoculated in duplicate into 5 per cent glucose of pH 2.5, 5.5, and 8.5 as these seemed to be more significant in the earlier tests. The number of cells for each tube inoculation was decreased to 200 per 0.02 cc to facilitate the colony count (Table III and Chart 2). An attempt was made in this group to learn whether dilution in 0.90 per cent NaCl solution damaged the cells seriously. After allowing these diluted cultures to stand for seventy two hours at a

temperature of 22° C. to 37° C., mostly the latter, F-40 and 157 grew poorly, while the others showed better growths in the twenty-four-hour subcultures. This may mean that more cells were inoculated (growth in aqueous NaCl solution), or that the living cells reproduced more rapidly following prolonged exposure to this inadequate medium.

TABLE II

ACTUAL CELL COUNT (HEMATOCYTOMETER) OF TWENTY-FOUR-HOUR 5 PER CENT GLUCOSE CULTURES. ORIGINAL INOCULATION APPROXIMATELY 100 CELLS PER CM.

pH	119	152	155	156
2.5	150	300	350	250
5.5	300			850
9.5	500	450	250	300

COLONY COUNTS AFTER ONE HUNDRED AND TWENTY HOURS IN POURED PLATES (INOCULATIONS FROM TWENTY-FOUR-HOUR 5 PER CENT GLUCOSE CULTURE). EACH 0.02 C.C. HAD 2,000 CELLS WITH ORIGINAL INOCULATION. AVERAGE OF DUPLICATE PLATES

pH	119	152	155	156
2.5	1,883	7,520	6,592	4,750
3.5	4,816	2,960	8,600	6,665
4.5	4,800	2,848	8,932	4,575
5.5	5,787	8,039	12,640	7,424
6.5	4,865	3,010	8,499	4,880
7.5	2,400	3,275	9,480	3,520
8.5	2,080	5,023	5,142	763
9.5	2	848	40	153

TABLE III

COLONY COUNTS AFTER FORTY-EIGHT HOURS IN POURED PLATES. EACH 0.02 C.C. HAD 200 CELLS WITH ORIGINAL INOCULATION INTO DILUENT. FIRST READINGS ARE AVERAGES OF DUPLICATE TUBES, INOCULATED IMMEDIATELY AFTER DILUTION. SECOND READINGS ARE COLONY COUNTS AFTER FORTY-EIGHT HOURS IN POURED PLATES, INOCULATION MADE FROM DILUENT AFTER IT STOOD WITH CELLS FOR SEVENTY-TWO HOURS

pH	F40	104	120	147	149	157
2.5	5	171	37	66	51	0
	0	420	1,400	800	1,248	0
5.5	12	500	58	272	193	2
	0	900	1,760	3,200	1,856	30
8.5	29	243	168	262	510	0
	13	1,600	668	1,040	1,200	0

*Glucose Broth Medium.*—Since the fungi grew poorly in a protein-free medium (which is in accord with mycologic reports), 0.5 per cent glucose in broth was employed, using the same technic, except that the number of cells

TABLE IV\*

COLONY COUNT AFTER TWENTY FOUR HOURS IN POURED PLATES (INOCULATIONS FROM TWENTY-TWO-HOUR GLUCOSE BROTH CULTURES). AVERAGE OF DUPLICATE TUBES. EACH 0.02 C.C. HAD 200 CELL, ORIGINAL INOCULATION

pH	F40	104	119	120	149	152	155	156	157
2.5	406	436	α	α	α	628	12	424	α
5.5	α	α	α	α	α	α	α	α	α
7.5	α	3,904	—	—	α	α	α	α	—
8.5	α	1,384	α	α	α	α	α	2,500	1,700

\* — = 0

α = innumerable

transferred was reduced from 2,000 to 200 per 0.02 cc of the inoculated medium. Strains F40, 120, 149, and 157 were observed in single cultures at pH 2.5, 5.5, and 8.5, while 104, 119, 147, 152, 155, and 156 were run at pH

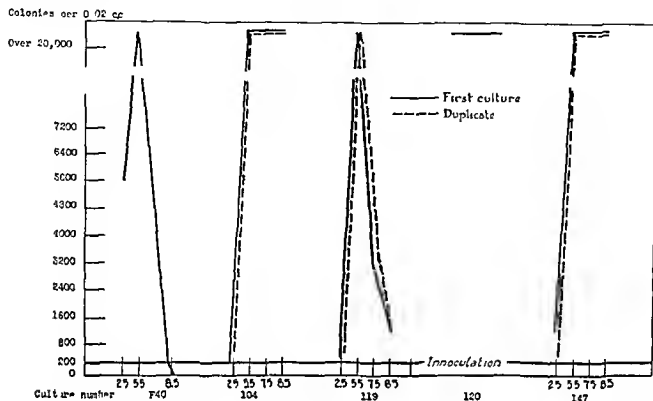


Chart 3—Colony count after twenty-four hours in poured plates Medium glucose broth

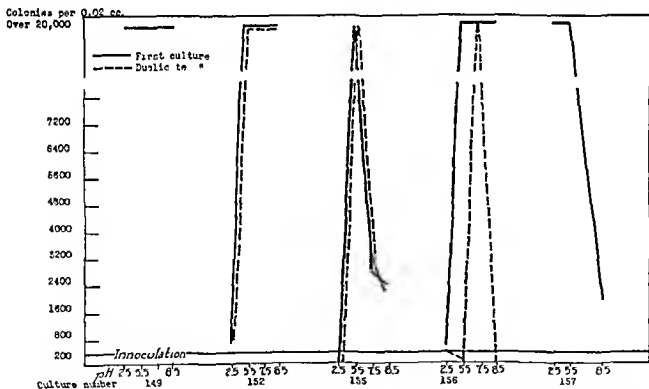


Chart 4—Colony count after twenty four hours in poured plates Medium glucose broth

2.5, 5.5, 7.5, and 8.5 in duplicate (Table IV and Charts 3 and 4, respectively). The growth was favorable for all strains at pH 5.5, while at the extreme ranges it was variable.

According to Tables II, III, IV and V, and Charts 1, 2, 3, and 4, the pH range from 2.5 to 8.5 represents the outside limits within which appreciable

cell growth occurred in these two media, even for the most versatile yeasts, while pH 5.5 was associated with uniformly good growth.

After cultivation of the inoculated media, the alkaline cultures became more acid, but the pH never changed more than 1.0. With media below pH 5.5 there was no consistent change. Tables V and VI show the time interval and the medium employed in the various tests, and are, perhaps, more significant than a detailed written explanation. In general, the optimum growth is less variable than Talice<sup>12</sup> reports. He states that the optimum pH growth range for *Cryptococcus* is 3 to 9, *Monilia albicans* 7 and *Saccharomyces* 3 to 4.

TABLE V

pH READINGS AFTER TWENTY-FOUR HOURS' CULTURE GROWTH IN 5 PER CENT GLUCOSE. AVERAGES OF DUPLICATE TUBES. SECOND READINGS ARE SEVENTY-TWO-HOUR READINGS

ORIGINAL pH	HOURS	F40	104	119	120	147	149	152	155	156	157
2.5	24	2.5	2.5	3.0	2.5	2.5	2.5	3.0	2.5	2.8	2.5
	72	2.5	2.5		2.5	2.5	2.5				2.5
3.5	24			4.0				4.0	4.0	4.0	
4.5	24			4.5				4.5	4.5	4.5	
5.5	24			5.5				5.5	5.5	5.4	
6.5	24			6.5				6.3	6.5	6.3	
7.5	24			6.6				6.7	6.7	6.7	
8.5	24	8.5	8.5	7.9	8.5	8.5	8.2	7.8	7.8	7.8	8.5
	72	8.2	8.2		8.0	8.2	8.2				8.2
9.5	24			9.2				9.2	9.2	9.3	

TABLE VI

pH READINGS IN GLUCOSE BROTH AFTER FORTY-EIGHT AND SEVENTY-TWO HOURS' GROWTH. AVERAGE OF DUPLICATE TUBES

ORIGINAL pH	HOURS	F40	104	119	120	147	149	152	155	156	157
2.5	48	2.8			2.8		2.8				2.6
	72		2.4	2.3		2.5		2.5	2.5	2.7	
5.5	72		5.6	5.5		5.4		5.5	5.2	5.5	
7.5	72		7.3	7.1		7.1		7.0	7.0	7.1	
8.5	48	7.4			7.8		8.0				7.8
	72		7.8	7.7		7.7		7.5	7.2	8.0	

# SUMMARY AND CONCLUSIONS

1. The mycotic organisms studied grew, generally, over ranges greater than are present in the human vagina.

2. One-half per cent glucose in broth was a better medium for the growth of these yeasts than 5.0 per cent protein-free glucose.

3. A pH 5.5 was preferable, but an absolute maintenance at this level was unnecessary for substantial growth of the fungi.

4. Strains 104, 120, 147, 149, and 153 grew well in pH ranging from 7.0 to 7.5, and will permit the study of the fungicidal effects of compounds insoluble, incompatible, or unstable in an acid medium.

5. Fungistatic action may be anticipated usually in some degree with a pH below 4.0 or above 7.5, while fungicidal action was evident at greater extremes.

6 It is suggested that the pathogenicity of a fungous strain may be evaluated by its ability to grow over greater pH ranges. This would suggest that 120 and 149 are more, 104, 147, and 152 less, and 119, 155, and 157 the least pathogenic. This idea is supported partially by the behavior of F 40, a proved nonpathogen.

7 It appears that either alkaline or acid instillations may be inadequate therapeutically because the extreme changes which they induce are only temporary.

8 Further research, which is in progress, concerns the general problem of the fungicidal potency of various agents.

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## FUNGICIDES\*

### II. IN VITRO TESTS WITH A NUMBER OF CHEMICALS ON YEASTLIKE ORGANISMS AND OTHER FUNGI

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THE clinical importance of infection by the pathogenic fungi has resulted in the publication of a large number of papers dealing with the etiologic agents, structures involved, and the therapy of such infections. The medications recommended for the treatment of vulvovaginal mycoses have been tested for ability to kill these organisms in only relatively few instances. The failure of gentian violet and alkaline douches<sup>1,2</sup> to bring about consistent and satisfactory results in vulvovaginal mycoses when patients cannot return for frequent treatments has stimulated a search for more effective agents.

The present investigation is concerned principally with the group of yeastlike organisms, the Monilias. A review of the work done previously in testing fungicides against the dermatophytes will be presented because of the possible application of the fungicides reported in this paper in the therapy of infections by dermatophytes.

Kolmer and Schamberg<sup>3</sup> tested the fungistatic and fungicidal powers of a number of chemicals against *Trichophyton rosaceum*, *Microsporon audouini* and *Achorion schoenleinii*. Iodine, mercuric chloride, and mercuraphen were found to be the most effective fungistatic or fungicidal agents.

Kingery and Adkisson<sup>4</sup> found an exposure for seventy-two hours to thymol, 1:6,250, iodine, 1:1,000, benzoic acid, 1:1,000, and salicylic acid 1:1,000, to kill all of several epidermophytos, trichophytos, achorions, a microsporon, a sporotrichum, and the organisms from two cases of blastomycosis.

Kadisch<sup>5</sup> smeared cultures of *Achorion gypseum*, *Trichophyton gypseum*, *Trichophyton depressum*, *Trichophyton acuminatum*, *Epidermophyton lanosum*, some thrush fungi, and a cryptococcus on linen cloth which was put in a 1 per cent solution of thymol in alcohol. The dermatophytes were destroyed by a five-minute exposure, but the yeastlike organisms survived for several hours.

Klarmann, Shternov, and Gates<sup>6-8</sup> report germicidal and fungicidal tests with a large number of chlorophenol derivatives. A number of the compounds tried were found effective in dilutions of 1:10,000 to 1:60,000 against *Trichophyton rosaceum*, *Achorion schoenleinii*, and *Monilia albicans* in ten minutes.

Yi<sup>9</sup> used *Epidermophyton rubrum*, *Epidermophyton interdigitale*, and *Trichophyton pedis* A and B as test organisms. He reported that these organisms

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were killed in five minutes by C-sellam's fuchsin dye 1 per cent thymol in 50 per cent alcohol 0.5 or 1.0 per cent benzoic acid and 0.5 per cent salicylic acid in 50 per cent alcohol.

Emmons<sup>6</sup> reports high phenol coefficients for sodium hypochlorite or iodine in alcohol or potassium iodide solution on *Trichophyton cup eum* and *Monilia albicans*. A number of chemicals—mercuric chloride phenyl mercuric nitrate formaldehyde malachite green gentian violet sodium ethyl mercuric thiocyanate hexylresorcinol thymol salicylic acid benzoic acid and others—gave low phenol coefficients.

McCrea<sup>11</sup> found that high dilutions of brilliant green and malachite green were lethal to *Trichophyton interdigitale* and *Epidermophyton rubrum* in one minute exposure.

Woodward Kingery and Williams<sup>7</sup> made tests on cephalosporium sporotrichum and *Monilia tropicalis*. An exposure of thirty minutes killed cephalosporium and sporotrichum in high dilutions of  $n$ -hexyl resorcinol  $n$ -hexyl ether of resorcinol chlorothymol and iodine. These and two additional compounds, 3,5-dibutyl phenol and mercuric chloride were also highly effective against *Monilia tropicalis*.

#### EXPERIMENTATION

Many compounds which have been used in vaginal therapy or recommended for the treatment of mycoses were tested under the conditions suitable for fungicidal tests and then under conditions more closely simulating those encountered in clinical use.

**Method.**—The strain used in the selection of effective compounds for further tests was a *Monilia albicans* (our Strain 94) which was isolated from a vulvovaginal mycosis. A 0.5 per cent glucose yeast infusion broth was inoculated from an active growth on a Sabouraud's glucose agar slant. After eighteen to twenty-eight hours' incubation at 37° C. the cells were suspended in broth by shaking and equal volumes of the suspension added to solutions of the chemicals in sterile test tubes. These tubes were shaken and five minutes after mixing (left at room temperature), one 3 mm. loopful of the mixture was transferred to sterile Petri dishes into which Sabouraud's agar was poured at once and the plates rotated in an attempt to obtain an even distribution of colonies. This method is essentially the same as the transfer method used by Ruehle and Brewer<sup>12</sup> and McCrea<sup>14</sup>. The plates were observed for several days unless growth occurred earlier. Growth was recorded in terms comparable to that of the control: 4 represents the maximum while 0 indicates no growth. The chemicals were used in concentrations which could be employed in vulvovaginal therapy. Anticipating the use of several acids in tests for fungicidal action the effect of pH was evaluated in preliminary work.<sup>15</sup> It was noted that even after twenty-four hours' exposure at various pH levels growth occurred at pH ranges of 3.5 to 5.5.

The preliminary test with *Monilia albicans* (Strain 94) served to indicate those reagents which appeared sufficiently promising for further study. These chemicals were then tested in the presence of human serum containing some



red blood cells. No antieoagulants were used in the preparation of the blood serum. The cell counts of these serums varied from 100,000 to 500,000 per c.mm. The serum was mixed with the solution of the chemical a few minutes before addition of the culture suspension. In most experiments the following volumes were used: solution of the chemical 0.5 c.c., serum 0.5 c.c., culture suspension 0.2 c.c. These components were mixed, and transfers made after five minutes as before. The chemicals which proved effective in these tests were then tried against several yeastlike organisms: a cryptococcus and four monilias isolated from vulvovaginitis cases, endomyces<sup>\*</sup> isolated from sputum, and *Saccharomyces cerevisiae*.

Besides the tests on the yeastlike organisms, a number of chemicals were tried against three other fungi: a plant pathogen, *Fusarium nivale*;† a dermatophyte, epidermophyton sp.;‡ and the common salmon pink, fluffy laboratory contaminant, monilia (in the botanical usage). The cultures were grown on Sabourand's glucose agar, and the growth suspended in saline. These suspensions were added to the solution of the chemical and treated as stated in the tests on Strain 94.

**Results.**—The results are given in Table I for all compounds tested against *Monilia albicans* (Strain 94). Relatively few chemicals proved effective even under these conditions.

The effective chemicals were then tried against Strain 94 in the presence of serum and cells. The various vehicles which were necessary to obtain solutions of the concentrations used are given in abbreviated form in Table II. Except alcohol, acetone, and water mixture which had little effect these vehicles were not fungicidal. Furthermore, serum and red cells did not inhibit the growth of the cultures as shown in controls. Under these conditions only five of the twenty-four substances killed all of the organisms present, while ten had little fungicidal activity.

Mercuric chloride, mercurophen, phenyl mercuric nitrate, chlorothymol, and iodine element preparations are the five chemicals which seemed to have therapeutic possibilities. The effectiveness of these five chemicals against several yeastlike organisms was next determined. The results are given in Table III. F 40 is a strain of *Saccharomyces cerevisiae*, 119 a cryptococcus, 104, 120, 155, and 156 were monilias, and 56924 an endomyces. With this group of organisms, chlorothymol and mercuric chloride were effective killing agents in a few instances, and yet had very little or no effect in others. Mercurophen failed to kill Strains 104 and 56924. Diluted Lugol's solution, colloidal, or "Safety Iodine," and phenyl mercuric nitrate killed all organisms.

The comparative resistance of four different fungi is given in Table IV. It is to be noted that these tests were not made with serum and cells present. Even under such favorable conditions for fungicidal action, only the colloidal,

NOTE: We wish to express our indebtedness to the following for cultures:

\*Dr. W. D. Stovall, Wisconsin Laboratory of Hygiene for the Endomyces culture number 56924.

†Dr. G. K. K. Link, the Department of Botany of the University of Chicago for the *Fusarium nivale*.

‡Dr. Max Obermayer, the Department of Dermatology of the University of Chicago for the epidermophyton sp.

TABLE I

 COMPOUNDS TESTED AGAINST *Monilia Albicans* (STRAIN 94) IN BROTH  
 Exposure Time Five Minutes ++++ Growth = to Control

	FINAL CONCENTRATION PER CENT	GROWTH		FINAL CONCENTRATION PER CENT	GROWTH
<i>Acids</i>					
1 Benzoic acid†	0.5	0	7 Pyrogallie acid	0.15	+++
2 Boric acid	2.0	+++	8 Pyroligneous acid	2.5	+++
3 Iodoacetic acid	0.5	++++	9 Salicylic acid	0.5	0
4 Lactic acid	5.0	++++	10 Sulfosalicylic acid	0.25	++
5 Perchloric acid	0.05	++	11 Trichloroacetic acid	0.05	++++
6 Picric acid	0.5	++++			
<i>Elements and Inorganic Compounds</i>					
12 Aluminum sulphate	2.0	+++	20 Potassium iodide	5.0	++++
2 Boric acid	2.0	+++	21 Potassium permanganate	0.05	++++
13 Copper sulphate	2.0	+++	22 Silver nitrate	0.5	++++
14 Gold chloride	0.05	++++	23 Sodium bicarbonate	20.0	++++
15 Iodine			24 Sodium borate	5.0	+++
Tincture iodine	0.5	0	25 Sodium fluoride	0.5	+++
Lugol's solution	0.5	0	26 Sodium hypobromite	0.14	+++
Glycerin solution	0.5	0	27 Sodium hypochlorite	0.1	0
"Safety iodine"	2.0	0	28 Sodium hypiodite	0.5	+++
16 Lead acetate	0.5	+++	29 Sodium perborate	0.5	++
17 Mercuric chloride	0.05	0	30 Sodium silicate	2.0	++++
18 Mercuric iodide	0.05	0	31 Sodium thiosulphate*	4.17	++++
5 Perchloric acid	0.05	++	32 Stannous chloride	2.0	+++
19 Potassium chlorate	0.25	++++	33 Zinc sulphate	2.0	++++
<i>Halogens and Halogen Compounds</i>					
34 Chloroform*	1.66	+++	18 Mercuric iodide	0.05	0
35 Chloramine	0.5	0	10 Potassium chlorate	0.25	++++
36 Chlorisothymol	0.1	0	20 Potassium iodide	5.0	++++
37 Chlorothymol	0.1	0	39 Sodium chloro orthophenyl phenate (Dowicide C)	0.05	0
38 Dettol*	2.08	++	25 Sodium fluoride	0.5	+++
15 Iodine			26 Sodium hypobromite	0.14	+++
Tincture iodine	0.5	0	27 Sodium hypochlorite	0.1	0
Lugol's solution	0.5	0	28 Sodium hypiodite	0.5	+++
Glycerin solution	0.5	0	40 Sodium tetrachlorophenate (Dowicide F)	0.05	+
"Safety Iodine"	2.0	0			
17 Mercuric chloride	0.05	0			
<i>Mercury, Silver, and Arsenic Compounds</i>					
41 Argyrol	5.0	++	45 Neosphenamine	1.0	++++
17 Mercuric chloride	0.05	0	46 Neosilvol	5.0	++++
18 Mercuric iodide	0.05	0	47 Phenyl mercuric chloride	0.05	+++
42 Mercurochrome	5.0	+++	48 Phenyl mercuric nitrate*	0.04	0
43 Mercurophen	0.05	0	29 Silver nitrate	0.5	++++
44 Merthiolate	0.05	+++	49 Stovarsol (Acetasone)	2.5	++++
<i>Dyes</i>					
50 Acriflavin	0.5	+++	42 Mercurochrome	5.0	+++
51 Brilliant green	0.5	+++	43 Mercurophen	0.05	0
52 Crystal violet	0.5	0	53 Metnphen	0.25	0
53 Gentian violet	0.5	++++	56 Methylene blue	0.5	+++
54 Mallophone	2.0	++	57 Pyridium	0.5	++
<i>Miscellaneous Organic Compounds</i>					
58 Acetaldehyde	0.75	++++	64 Phenol	0.5	++++
59 Anilin ammonium succinimide	0.5	++++	65 Potassium antimony tartrate	0.5	++++
60 Cresol	0.5	0	66 Quinine dihydrochloride	5.0	++++
61 Formal	0.05	+++	67 Resoreinol	1.0	++++
62 Furfuraldehyde	2.0	++++	63 Thymol	2.5	0
63 Hexylresorcinol	0.05	0	69 Urotropin	0.5	++++

\*Tested with serum and cells present.

†Compounds are numbered in order of their first appearance in the table.

TABLE II

TESTS WITH *Monilia Albicans* (STRAIN 94) IN BROTH WITH HUMAN SERUM AND RED CELLS ADDED

Exposure Time Five Minutes. +++ Growth = to Control

CHEMICAL	*VEHICLE	CLINICAL CONCEN- TRATION PER CENT	FINAL CONCEN- TRATION PER CENT	GROWTH AFTER EXPOSURE 5 MIN.
1. Benzoic acid	AGW	0.5	0.2	++
9. Salicylic acid	AW	1.0	0.4	+
14. Gold chloride	W	0.1	0.04	++++
15. Iodine				
Lugol's solution	WKI	1.25	0.5	0
Tincture iodine	AW	1.75	0.7	0
"Safety Iodine"	AW	1.0	0.4	+
	W	4.0	1.6	0
	W	1.0	0.4	+++
Glycerin solution	G	1.0	0.4	+++
17. Mercuric chloride	W	0.1	0.04	0
18. Mercuric iodide	WKI	0.1	0.04	+
27. Sodium hypochlorite	W	0.24	0.1	+
31. Sodium thiosulphate	W	10.0	4.17	++++
34. Chiniofon	W	4.0	1.67	+++
35. Chloramine	W	1.0	0.4	+++
36. Chlorisothymol	W	0.5	0.2	++
37. Chlorothymol	AW	1.0	0.4	0
	AGW	0.5	0.2	0
	AGW	0.2	0.08	+++
	W	5.0	2.08	++
38. Dettol				
39. Sodium chlor-orthophenylphenate (Dowicide C)	W	0.24	0.1	+
40. Sodium tetrachlorphenate (Dowicide F)	W	0.24	0.1	++++
43. Mercurophen	W	0.2	0.08	0
	W	0.1	0.04	+++
47. Phenyl mercuric chloride	Walk.	0.1	0.04	++
48. Phenyl mercuric nitrate	AWalk.	0.1	0.04	0
49. Stovarsol (Acetarsone)	W	5.0	2.0	+++
	W	2.0	0.8	++++
52. Crystal violet	W	1.0	0.4	+
55. Metaphen, tincture	A Ac W	0.2	0.08	+++
	AW	0.13	0.05	++++
60. Cresol	W	1.0	0.4	+++
63. Hexylresoreinol	W	0.1	0.04	+++
68. Thymol	AGW	0.25	0.1	+++

\*alk., Alkali  
A, Alcohol  
Ac, Acetone

G, Glycerin  
KI, Potassium iodide  
W, Water

TABLE III

TESTS WITH A NUMBER OF YEASTLIKE ORGANISMS IN BROTH WITH HUMAN SERUM AND RED CELLS ADDED

Exposure Time Five Minutes. +++ Growth = to Control

	FINAL CONCENTRATION PER CENT	ORGANISMS TESTED						
		F40	140	119	120	155	156	56924
Chlorothymol	0.21	0	+++	++	+	0	++	0
Iodine								
Lugol's solution	1.03	0	0	0	0	0	0	0
"Safety Iodine"	1.64	Not run	0	0	0	0	0	0
Mercuric chloride	0.04	+	+++	++++	++	0	++	++++
Mercurophen	0.08	Not run	++	0	0	0	0	+++
Phenyl mercuric nitrate	0.04	0	0	0	0	0	0	0

or "Safety Iodine" and sodium chlor orthophenylphenate (Dowicide killed all four fungi. There was considerable difference in resistance in the four types of fungi, but in general, *Monilia albicans*, Strain 94, was more resistant.

TABLE IV  
COMPARATIVE SUSCEPTIBILITY OF FOUR FUNGI TO VARIOUS CHEMICALS  
Exposure Time Five Minutes ++++ Growth = to Control

CHEMICAL	FINAL CONCENTRATION PER CENT	<i>Monilia Albicans</i> STRAIN 94	<i>Fusarium Nivale</i>	<i>MONILIA SP (SITOPHILA TYPE)</i>	<i>EPH MOPH S</i>
Benzoic acid	0.5	0	0	0	
Formaldehyde	0.15	+++	0	++	
Furfural	2.0	++++	+	++++	
Iodine					
"Safety Iodine"	2.0	0	0	0	
Iodoacetic acid	0.5	+++	0	+	
Mercuric chloride	0.03	0	Not run	Not run	
Mercuriophen	0.05	0	0	+++	
Merthiolate	0.05	++++	0	0	
Phenol	0.5	++++	++	++++	Ne
Picric acid	0.5	++++	0	0	
Pyrogallol acid	0.15	+++	++	+++	Ne
Salicylic acid	0.5	0	0	0	
Sodium chlor orthophenylphenate (Dowicide C)	0.05	0	0	0	
Sodium hypochlorite	0.1	0	0	+	
Sodium tetrachlorophenate (Dowicide F)	0.0	+	++	+	

The reviewed literature indicates that the following chemicals have fungicidal activity toward dermatophytes and monilias: iodine, mercuric chloride, mercuriophen, thymol, n-hexylresorcinol, salicylic acid, benzoic acid, brilliant green, malachite green, and chlorophenol derivatives. These compounds, with the exception of malachite green and the chlorophenol derivatives, were tested under conditions not radically different from those employed by the author mentioned. Under these conditions the results in the previous reports were confirmed. Malachite green, brilliant green, but more often crystal violet and gentian violet have been recommended for mycotic infections. In tests performed in broth only crystal violet killed *Monilia albicans* (Strain 94) in five minute exposure time.

In none of the studies reviewed were the organisms tested under conditions approaching *in vivo* states. In an attempt to approach the *in vivo* environment, human serum and blood cells were added. Whereas the previous tests would have left the impression that a number of active fungicides had been included in the list of chemicals studied, the test with serum and the present indicated that only a very few of these chemicals possessed fungicidal properties under conditions simulating those in obstetric and gynecologic therapy. A number of the remaining chemicals would kill organisms if exposed for a longer period of time, however in therapy within body cavities such as the vagina, it is difficult to obtain prolonged contact between medicament and the entire surface. The chemicals showing the most promise for clinical application are iodine as Lugol's solution (1:2) or a colloidal

preparation ("Safety Iodine") and phenyl mercurie nitrate 1 to 1,000. The 1:2 Lugol's solution has produced irritation in some cases, and in them a 1:4 dilution was used. The "safety iodine" was not irritating when properly suspended and when no deterioration had taken place. The phenyl mercurie nitrate was dissolved in 15 per cent alcohol and 2 per cent 0.1 N sodium hydroxide.

The assumption is not uncommon that a compound which has fungicidal action may be active against fungi in general. From Table IV it may be seen that this view has little support when formaldehyde, a reputedly good fungicide, had very little effect on *Monilia albicans*, Strain 94. In addition to formaldehyde, furfural, merthiolate, and picric acid showed extreme variability in their power for killing the different species. The difficulty in finding a universal fungicide is apparent especially when even the closely related yeastlike organisms show moderate variability in their susceptibility to fungicides (see Table III).

#### CONCLUSIONS

1. Employing a loop transfer method of testing, a considerable number of chemicals were capable of killing *Monilia albicans* (Strain 94) in five minutes exposure in concentrations clinically usable.

2. In the presence of human serum and blood cells, mercurie chloride, mercurophen, phenyl mercurie nitrate, chlorothymol, dilute Lugol's solution, and colloidal iodine killed all organisms of Strain 94.

3. Seven additional organisms, a saccharomyces, a cryptococcus, four monilias, and an endomyces were subjected to the chemicals enumerated in two, under similar experimental conditions. It was noted that only phenyl mercurie nitrate, the diluted Lugol's solution, and the colloidal iodine preparation were effective against the organisms tested.

4. Tests for killing power were made with 15 compounds on 4 unrelated fungi, 2 of medical, and 2 of botanical interest. The results show obvious differences in strain susceptibility.

5. Even within a genus, considerable difference may exist in susceptibility to fungicides.

6. Before any compound can be asserted to be a fungicide, it must be tested against many varieties of organisms for which it is to be used therapeutically.

7. The existence of an universal fungicide suitable for clinical use is extremely doubtful.

8. The more promising compounds are being tried in clinical studies of vulvovaginal mycoses.

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# THORACIC DUCT LYMPH PRESSURE IN CONCRETIO CORDIS\*

## AN EXPERIMENTAL STUDY

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PICK'S disease or concretio cordis was produced in two dogs by the introduction of aleuronat into the pericardial cavity. This substance is irritating, and it results first in the formation of fluid in the pericardial cavity and later in the fusion of the pericardium and epicardium. The venous pressure

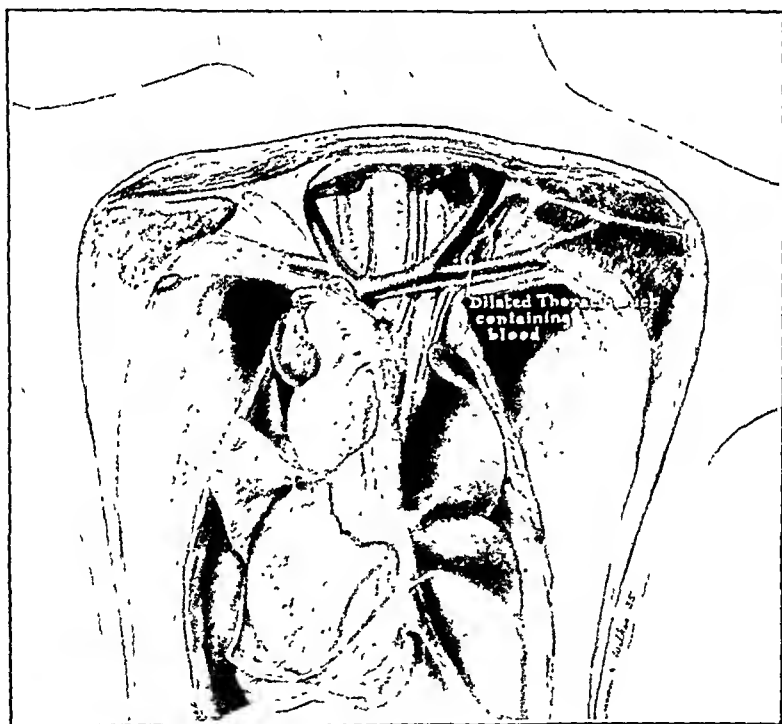


FIG. 1.

rises and fluid accumulates in the peritoneal cavity. Similar changes were produced by Beck and Griswold† by the introduction of Dakin's solution into the pericardial cavity.

Three weeks following the introduction of aleuronat into the first dog, the pressure in the external jugular vein was 120 mm. water and that in the

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†Beck, C. S., and Griswold, R. A.: Pericardectomy in the Treatment of the Pick Syndrome, *Arch. Surg.* 21: 1064, 1930.

femoral vein was 140 mm water. Under morphine narcosis, the cerebrospinal fluid pressure was found to be 240 mm water. Under ether anesthesia, the thoracic duct which was markedly dilated was exposed in the neck. Blood was present in the duct for a distance of approximately 1 cm peripheral to its entrance into the vein. By inserting a needle into the duct and allowing salt solution to flow in the pressure in the duct was found to be 150 mm water while that in the subclavian vein was 165 mm water. The animal was killed and a typical instance of concretio cordis was demonstrated. Twelve hundred cubic centimeters of fluid were present in the peritoneal cavity and an exudate was found on the surface of the liver. A drawing of the autopsy specimen is shown in Fig. 1.

In the second experiment, the animal appeared ill seventeen days following the introduction of alcuronat. The pressure in the external jugular vein was 155 mm water and that in the femoral vein was 175 mm water. The cerebrospinal fluid pressure was 200 mm water. Using ether anesthesia, a markedly dilated thoracic duct was exposed. A small amount of blood could be seen during expiration in the duct at its entrance into the vein. The pressure in the thoracic duct was found to be 200 mm water and that in the subclavian vein to be 175 mm water. Following the removal of the needle from the duct, a pulsating stream of lymph shot out through the needle hole during each expiration. Several hundred cubic centimeters of lymph escaped during the thirty minutes that the duct was exposed. The incision was closed and the animal died unexpectedly two days later. There were 1100 cc of blood tinged fluid in the pleural cavities and 90 cc of thin yellowish fluid in the peritoneal cavity. The pericardium and epicardium were fused and thickened. There was an exudate covering the liver.

#### SUMMARY

Concretio cordis was produced in dogs by the introduction of alcuronat into the pericardial cavity. The thickening and fusion of the pericardium and epicardium were associated with a dilatation of the thoracic duct, with a lymph pressure elevated to approximately the same extent as the venous pressure, and with a small amount of blood in the proximal end of the duct. No conclusions are drawn as to the part if any which the elevated lymph pressure plays in the accumulation of fluid in the serous cavities.



## THE DIURETIC ACTION OF MERCUPURIN\*

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EVER since the rediscovery of the use of mercury as a diuretic by Jendrassik<sup>1</sup> in 1886, mercury has been an important adjunct in the treatment of cardiac dropsy. Jendrassik, believing a case of dropsy to be of syphilitic origin, used calomel and jalap in the treatment. So effective were the results that further investigation revealed that it was the calomel which was the diuretic agent. The use of mercury in edema was given further impetus by the discovery and introduction of organic mercury compounds in 1917. This time Zieler<sup>2</sup> introduced novasurol as an antisyphilitic. The diuretic effect of this drug, however, was described by Saxl and Heilig<sup>3</sup> in 1920. In 1924 salyrgan was introduced by E. Bernheim<sup>4</sup> as another organic mercury diuretic.

Novasurol, merbaphen, is the double salt of sodium mercuriphenyl oxyacetate with diethylbarbituric acid and contains 33.9 per cent mercury directly united to the benzene ring. Salyrgan, mersalyl, on the other hand is an aromatic compound containing 39.6 per cent mercury in the form of the hydroxy mercuric radical, HgOH, firmly attached to the end of a side chain. Mercupurin, introduced in foreign literature as novurit, is the third and newest of the group of organic mercury diuretics. This compound is the sodium salt of trimethylelopentanedicarboxylic acid—methoxymercuriallylamide—theophylline.

Following the animal experiments of Issekutz and Vegh<sup>5</sup> it was found that mercupurin was less toxic than novasurol and salyrgan and that it had greater diuretic effects than its predecessors. Clinical tests by Berger, Fazekas and Galgoczy<sup>6</sup> in Hungary confirmed the animal experiments of Issekutz and Vegh.

Further clinical tests by Popper<sup>6</sup> and Hahn<sup>7</sup> agree that mercupurin is an efficacious mercury diuretic without toxicity.

Since there has been no confirmation of these reports in American literature up to the present time, we have undertaken to establish the value of this drug in the treatment of cardiac edema. We have confined our experiments to the use of mercupurin intravenously in 2 c.c. doses. For the most part the patients studied were placed on the usual cardiac routine for several days before the drug was administered. This was particularly true of the earlier cases. The routine consisted of bedrest, digitalis, ammonium chloride, and restricted fluids. When it was noted that the limit of renal output was established, mercupurin was given. The daily output of urine was recorded as well as the daily weight of the patient. We felt that the weight was a

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TABLE I

NAME	AGE	SEX	DIAGNOSIS	WEIGHT IN LB FIRST DAY OF INFECTION	FIRST 24 HR		SECOND 24 HR		THIRD 24 HR		URINE BEFORE MERCUPURIN				URINE AFTER MERCUPURIN				MAXIMUM WEIGHT LOSS PER INJEC- TION (TR)
					WEIGHT IN LB	HR	WEIGHT IN LB	HR	WEIGHT IN LB	HR	ALB	CASTS	W B G	R B G	ALB	CASTS	W B G	R B G	
1 M B	31	m	Mitral stenosis	137	1300	161	2200	156	1200	1564	+++	Occ	Occ	Rare	Rare	Rare	Rare	Rare	4
2 G E	71	m	Hypertension	171	2300	149½	2150	153	2100	148	Tr	None	None	None	None	None	None	None	2½
3 N H	37	m	Rheumatic	174	2000	147	1800	150½	1532	152	+++	Occ	Occ	None	None	Occ	None	None	4½
4 G W	60	m	Hypertension	154½	2600	154½	1550	146	1100	144½	+++	Many	None	Rare	None	None	None	None	10
5 E H	63	m	Coronary	(1) 163½	2600	154½	1850	136	900	136	+++	Occ	Few	None	+++	Occ	None	None	22
6 M M	44	f	Aortic insuf- ficiency syphilitic	(2) 158	4600	137	3000	136½	900	136	+++	None	None	None	+++	None	None	None	10
				(1) 245	1360	237	450	236	1250	235									
				(2) 210	4060	208	2400												
				(3) 185	2500	173	1850	176	550	177½									
				(4) 178	900	173	2150	174	425	173½									
				(5) 179	1000	177	850	178½	625										
7 M C	55	f	Hypertension	168	2450	146	1450	145	900	155	++	Rare	Rare	None	+	Occ	Few	None	1*
8 B C	50	f	Hypertension	(1) 164	2370	1404	4150	145	700	147	+	Occ	Occ	Rare	None	None	Rare	Rare	
				(2) 151½	2470	131½	2350	131	250	143					+++	Many	Many	Many	20½
9 F M	50	m	Coronary	156½	3300	140	3800	134	3100	132½	++	None	None	None	None	None	None	None	24
10 E S	41	m	Mitral stenosis	182	6300	164	5900	155	1300	157	++	None	Few	None	None	None	Rare	Rare	27
11 J H	54	m	Coronary	142	4350	129	3700	127	1500	124	+	None	Few	None	None	None	None	None	19
12 L M	60	f	Coronary	(1) 164	4015	171	3210	151	600	171	++++	Occ	Occ	Rare	++++	Occ	Occ	Occ	
				(2) 152	230	144	2000	150	500	146½									
				(3) 160	5150	146	2750	146	1150	152					++++	Occ	Occ	Rare	14
13 F W	62	f	Coronary	157	1800	155	2975	151	1150	152	+++	Occ	None	Rare	+++	Occ	Occ	None	34
14 C S	50	m	Hypertension	168	2850	159	2100	160	1400		++++	None	None	Rare	Tr	Rare	Rare	None	9
15 H B	73	m	Coronary	(1) 172	5000	167	2400	165	950	166	+	Rare	None	None	Lethal	exodus	None	None	8
				(2) 172	1000	165	900	164	166						Tr	Rare	Rare	None	
				(3) 167	1700	162	5100	164	950	163									
16 E G	69	m	Hypertension	136	2300	159	700	112	1200	125	+++	Rare	Occ	None	Tr	Rare	Rare	None	11
17 G B	68	m	Hypertension	134	1000	116	1750	112	1400	108	+++	None	Occ	None	Tr	Occ	None	None	26
18 O M	43	m	Hypertension	(1) 216	4950	202	4000	200	3000	200	Tr	None	None	None	None	Occ	Occ	None	20
				(2) 190	3400	173	3500	176	700	177									
				(3) 207	7500	191	4700	196	1100	196					Tr	Many	Occ	None	25
19 A P	76	m	Empysem.	208	600	199	1500	192	1100	183	Tr	None	None	None	+	None	None	None	12
20 A R	33	m	1 B pericarditis	(1) 140	2000	128	4000	128	1450	132	+	None	None	None	+	None	None	None	21
				(2) 140	3400	134	1400	131	200	129					+	None	None	None	30
21 F W	33	f	Hypertension	148	6350	123	1120	127	620	129	None	None	None	None	+	None	None	None	21
22 I M	42	m	Hypertension	163	4700	133	1600	134	760	136	++	None	None	None	+	None	None	None	30

more accurate index of the fluid loss than the estimate of the urine, as in many instances patients were incontinent.

Twenty-two patients were studied and a total of thirty-five intravenous injections of mereupurin were given. No nephritis, gastro-enteritis or untoward reactions of any kind followed the use of the drug. The following protocol illustrates the type of patient treated and the fluid and weight loss.

#### SUMMARY

1. Mercupurin was used intravenously in 2 c.c. doses in twenty-two individual cases.
2. Thirty-five injections were administered.
3. Mereupurin proved to be an effective diuretic.
4. No ill results were noted.
5. Weight losses ranging from 3 to 30 pounds per injection were attributable to the diuretic action of the drug.

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# LABORATORY METHODS

## PREPARATION OF THE KRUEGER UNDENATURED BACTERIAL ANTIGENS\*

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### INTRODUCTION

IN THE past, attenuation of various virus antigens by physical or chemical means or by propagation in heterologous species has contributed greatly to the success of protective artificial immunization. On the other hand bacterial pathogens and their products have been subjected to such a variety of conditions and methods of treatment in their preparation for use as antigens that almost all degrees of success of protective artificial immunization have been obtained at some time or other.

For example, Pasteur's chicken cholera vaccine was considerably attenuated in virulence by age and presence of accumulated metabolic substances, however, it was highly immunizing and no doubt much native brilliant substance was present in the form of organisms which remained viable. Heat treated typhoid vaccine may be mentioned as another immunizing antigen of well-known effectiveness. In this instance, while the vaccine comprises killed microorganisms it is nevertheless quite useful in human immunization, and may be cited as a strong antigen of much more than average stability against heat. A third antigen, or rather series of antigens, is of interest, namely, that derived from the diphtheria bacillus. In this instance administration of highly antigenic toxin is made practical by modifying it with antitoxin or attenuating it with formaldehyde, and subsequently by rendering the product of this latter treatment rather insoluble to allow of longer tissue stimulation. Units of measurement of antigenicity and corresponding immunity are so well known in this instance, that a high degree of precision is attained in practical immunization. A fourth type of antigen, namely bacteriophage antigen, has been the subject of an enormous amount of work. A useful kind of devitalization of some bacteria may be brought about by the use of bacteriophage, and lysate antigens so prepared may have more merit in some instances than corresponding heat treated antigens. We do not consider the direct participation of bacteriophage itself, but regard it as a means of producing solutions of bacterial substance.

Increasing interest has been exhibited in the last few years in what actually happens to bacillary substance in preparing bacterial antigens. Perhaps where viable bacteria can be used or where the antigen, be it heterophile or not, is unusually heat stable, or where the technique of antigen preparation is so exactly reproducible due to ease of determination of units of activity, the prob-

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lem is not so urgent. However, many bacteria are left on the list, artificial immunity to which presents much room for improvement and an obvious need for more useful antigens.

The older work of Obermeyer and Pick<sup>1</sup> on denaturation of protein and corresponding immunologic responses, and the more recent contributions to this subject by Anson and Mirsky<sup>2</sup> have centered attention in vaccine preparation upon control of denaturation processes commonly occurring in bacterial antigen preparation.

We have experimented with devitalization of bacteria with the noncoagulating antiseptic merthiolate<sup>3</sup> as an improvement over heat treatment of bacteria in the preparation of more nearly natural or native antigens. More recently Krueger and his associates<sup>4</sup> have described experimental methods for mechanical devitalization and fragmentation of bacterial cells and subsequent merthiolate preservation of the antigen causing practically no denaturation of protein. In this report we wish to describe experimental preparation of the Krueger undenatured bacterial antigen ("U.B.A.") and some laboratory results of assaying different preparations of such bacterial substances.

#### EXPERIMENTAL

We have prepared, on an increasingly large scale, undenatured bacterial antigens from *H. pertussis*, from several other respiratory bacteria, including pneumococci, streptococci, and *H. influenzae*, from staphylococci, from colon and typhoid bacilli, and from gonococci.

*A. Bacterial Culture and Culture Media Utilized.*—*H. pertussis*: freshly isolated strains grown on human or sheep blood (25 per cent) Bordet agar were utilized up to six months of age. All of these cultures were smooth, hemolytic, agglutinated well with "Phase 1" antiserum, and as far as could be determined fulfilled the characteristics of S strains as recently defined by Shibley.<sup>5</sup> Each lot of pertussis U.B.A. was prepared in the following way: Not less than five nor more than twenty-five cultures were utilized as seed in the form of a forty-eight-hour growth on large Bordet blood agar tubes. The growth from each large tube was suspended in 10 c.c. Bordet "potato juice" and planted in 2.5 c.c. amounts to four Bordet blood agar blade bottles. At first a lot comprised twenty-four such blade bottles, but later from a practical standpoint the size of each lot was increased gradually up to four hundred blade bottles. Fig. 1 shows the appearance and method of handling such a lot of bottles. After incubation at 35° C. for forty-eight hours, the pure culture growths were washed from the medium with sterile Loeke-Ringer solution.

*Pneumococci and Streptococci*: The pneumococcus cultures included Types 1, 2, 3, and a member of Cooper's Type 4, and also a well-known broad heterophile antigen strain "DRI" originally coming from the Neufeld Type I strain. The first four cultures were mouse passaged once weekly and kept in rabbit blood beef infusion broth, while the DRI culture was simply cultivated in this medium without passage. The streptococci included at first representative hemolytic, viridans, and indifferent strains used to produce regular bacillary vaccine in this laboratory. Later two highly virulent mouse passaged streptococcus strains were added which regularly killed mice in doses of  $10^{-7}$  or  $10^{-8}$

c.c. Each lot of pneumococcus or streptococcus U.B.A. was prepared by planting rabbit blood broth seed cultures into lots comprising usually sixteen large three-gallon bottles of freshly made warm beef infusion broth to which 0.1 per cent dextrose was added after sterilization, and growing these at 37° C for



Fig 1.

twenty-four hours. The bacteria were recovered from these massive cultures through the use of a Sharples centrifuge, and suspended in Locke-Ringer solution.

*H. influenza.* Four laboratory strains were utilized at the start of the work. Later two smooth strains, representative A and B types supplied by

Dr. Margaret Pittman, were included, and subsequently an Indianapolis strain freshly isolated from a case of pharyngitis, and having definite virulence in pure culture for mice, was added. These organisms were grown twenty-four hours in cleared chocolate broth at 37° C. and the bacteria were recovered with a Sharples centrifuge and suspended in Locke-Ringer solution.

*Colon and Typhoid Bacilli:* Bacillary vaccine laboratory strains were used, and each lot of undenatured bacterial antigen was prepared by planting broth seed culture to blake bottles of dextrose beef infusion agar and growing these at 37° C. for twenty-four hours. The bacterial growth was washed from the agar and suspended in sterile Locke-Ringer solution.

*Gonococci:* Bacillary vaccine laboratory strains were grown forty-eight hours on blake bottles of testicular agar as for regular vaccine preparation. The bacterial growth was washed from the agar and suspended in sterile Locke-Ringer solution.

*B. Subsequent Treatment of All Suspensions of Bacteria in Locke-Ringer Solution Immediately on Recovery From Culture Medium.*—These suspensions were rapidly sedimented at high speed on an International centrifuge, the supernatant fluid discarded, an additional Locke-Ringer solution added, and the sediment resuspended. This process of washing was again repeated, and the final bacterial sediment was made up to a thick consistency with additional Locke-Ringer solution.

At this point the washed suspensions were ground according to the Krueger technic in glazed ball mills about three-quarters full of three-eighth-inch stainless steel balls, rotating at speeds which may vary from 30 to 120 revolutions per minute. Grinding was continued for from sixteen to twenty hours at which time nearly all cells were fragmented and very few intact bacterial cells were present as shown by stained smears. The solutions then were spun lightly on an International centrifuge to remove coarse particles and thereupon were ultrafiltered.

Filter membranes were relatively nonadsorptive and were prepared from Whatman number fifty filter papers impregnated in the usual way with a 4.5 per cent solution of acetic cotton. Ultrafilters supporting membranes 7 cm. in diameter, and holding a volume of about 200 c.c., were used at first. Later, larger ultrafilters, supporting membranes of 18.5 cm. in diameter and holding a volume of about 2,000 c.c., were used to accelerate filtration. Fig. 2 shows the appearance of these ultrafilters.

Denaturation of bacillary protein during the grinding procedure was prevented by use of buffered solution, slow speed of the ball mills avoiding friction heat, and conducting the grinding in quarters not subjected to overheating. Denaturation during ultrafiltration was avoided by rapidity of procedure thus preventing prolonged exposure of the protein substrate to viable bacteria which may have escaped grinding, and conducting the ultrafiltration in laboratory quarters also not subjected to overheating. The ultrafiltrates were preserved with merthiolate 1:100,000, instead of the phenoloid preservatives. This concentration was used since it was well beyond the denaturing concentrations of this chemical for native bacillary protein in such pure state.

C. *Standardization of U.B.A. Ultrafiltrates.*—Micro Kjeldahl nitrogen determinations were made upon each separate lot of the various U.B.A. preparations. These values varied mainly from about 5 mg. to about 75 mg. nitrogen per 100 c.c. of ultrafiltrate depending on the species of microorganism used, and the density of the suspension subjected to grinding. Two U.B.A. (staphylococcus and pneumococcus) lots were made which contained over 100 mg. nitro-

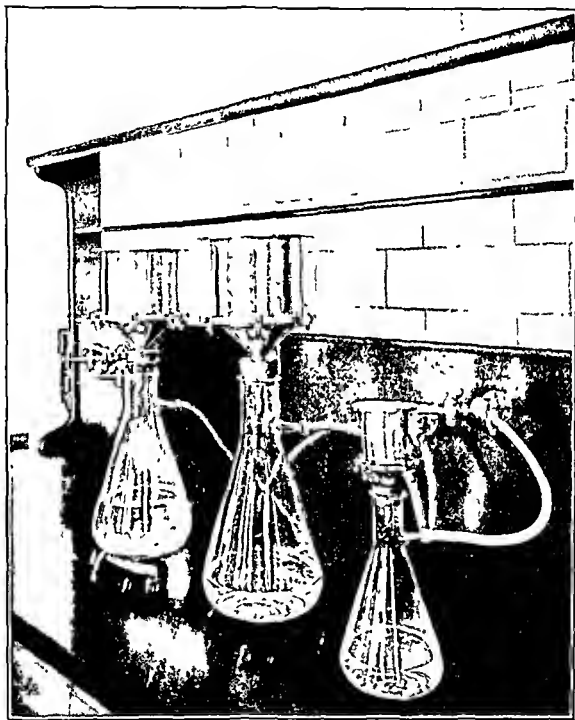


FIG. 2

gen per 100 c.c., while the average preparations contained about 25 mg. nitrogen per 100 c.c. (see Table I).

At the suggestion of Dr. A. P. Krueger we have tested these U.B.A. ultrafiltrates further for undesirable denatured protein by isoelectric precipitation at about pH 4.5. About 1 per cent of such antigens show traces of denaturation and these have been discarded as unsatisfactory for further experimental use.

Utilizing these two criteria of excellence, preliminary trial showed that human treatment could most satisfactorily be conducted with pertussis U.B.A.



TABLE I

UNDENATURED BACTERIAL ANTIGENS (U. B. A.): LABORATORY LOTS OF VARYING SIZE AND CONCENTRATION

U. B. A.	AMOUNT MEDIA (BLAKE BOTTLES OR LITERS OF BROTH)	PREPARATION NUMBER	VOLUME (CUBIC CENTIMETER)	NITROGEN (MG.M.) PER 100 C.C.
Pertussis	100 bottles	B-2947	225	13.48
Pertussis	100 bottles	B-2948	240	10.41
Pertussis	172 bottles	B-2955	170	41.08
Pertussis	150 bottles	B-2958	280	26.64
Pertussis	150 bottles	B-2967	375	14.44
Pertussis	300 bottles	902009	800	16.80
Streptococcus	30 liters	B-2946	100	69.68
Staphylococcus	25 bottles	B-2888	70	141.92
Pneumococcus	160 liters	902812	2500	19.76
Gonococcus	300 bottles	902005	425	19.5

containing 10 mg. nitrogen per 100 c.e., while optimum concentration of nitrogen in "respiratory," staphylococcus, streptococcus, and gonococcus U.B.A. was 5 mg. per 100 c.e. These undenatured bacterial antigens have been supplied to a large number of clinical investigators for evaluation. Some of their results have appeared in the literature, and further reports are in progress. Suffice it to mention here that their use appears definite and readily demonstrable, so far as such studies have progressed and their widest respective clinical use has been in the treatment of pertussis, chronic paranasal sinusitis and other respiratory conditions.

*D. Comparative Properties of U.B.A.*—As compared to conventional vaccine, U.B.A. is quite free of undesirable bacillary metabolic products, and culture media ingredients, and also is relatively atoxic. In terms of numbers of original bacteria represented, a much greater amount of native U.B.A. can be conveniently administered than can with the conventional vaccine. Reactions from several multiples of an optimum human dose comprise slight local stiffness and sensitiveness to pressure. There is an unusual freedom from general systemic reactions of any sort. Theoretically U.B.A. offers a good substitute for living vaccine. It comprises fragmented cells and native bacillary protein solution uncontaminated with so-called nonspecific substances, and unchanged by the technique of preparation. It is a mild antigen as compared with many irritating vaccines. The sum total of immunologic response is not dissipated against associated split products and denatured proteins following the use of U.B.A. Antibody response in animals has been only partly investigated, and although agglutinins and other antibacterial bodies are produced, they are not spectacularly high titered, but in some instances appear somewhat earlier than usual. Rapidity of therapeutic action of pertussis U.B.A. suggests some form of desensitization or altered tissue reactivity as the likely mechanism of action, rather than immunization as commonly thought of. Also, since antibody such as agglutinin does not regularly parallel immunity, either active or passive, the titer of serum antibody following U.B.A. treatment is not an accurate indicator of resistance. It follows that precision laboratory tests of the degree of resistance incited by U.B.A. are yet to be devised.

## SUMMARY

By utilizing carefully selected cultures as the starting point, and the application of large scale bacteriologic procedures, we have prepared the Krueger UBA in increasing large quantities for clinical use. The Krueger UBA is quite free of the degradation products associated with conventional vaccine.

These antigens have been standardized to desired nitrogen content when finished, and are preserved with merthiolate. Accidental or unforeseen denaturing effects occurring during preparation are detectable in the final laboratory examination of each antigen. Through elimination of any antigen showing a trace of denaturation as witnessed by isoelectric flocculation of denatured protein, a supply of native bacterial antigen is assured. UBA in quantity has been produced mainly from *H. pertussis*, several bacteria associated with upper respiratory disease, pyogenic cocci, and the gonococcus.

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## A MORE SENSITIVE COMPLEMENT FIXATION TEST FOR GONORRHEA\*

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THE classical complement fixation test for gonorrhea has not given satisfactory results. By the customary gonorrhea fixation test, it is seldom that clear cut positive and negative reactions are obtained.<sup>1</sup> When attempts are made to obtain definite positive results in known cases, a number of indefinite reactions are obtained upon patients who are undoubtedly negative. For these reasons, the test is carried on by few laboratories.

The complement fixation test for gonorrhea presented here is much more sensitive in the determination of immune substances than existing tests. Work with children in institutions indicates that it does not give false positive or indefinite results except in meningitis.

In addition, it is truly quantitative because it determines accurately the amounts of complement absorbed nonspecifically by every specimen. Furthermore, it takes into account that quantitative relations do not exist between reaction tubes which contain varying amounts of serum. In this test, the specific fixation by the patient's serum is read in conjunction with the amount of non-specific absorption of complement, and the same amounts of serum are used in the control and each of the reaction tubes.

The method presented here requires nothing more than a readjustment of the quantities of reagents used and a new interpretation of readings. The reasons behind the changes are important to complement fixation work in general, and to the gonorrhea fixation in particular.

The question has often been raised as to whether complement fixing bodies were developed in gonorrhea infections in sufficient quantities for practical diagnosis. Because of the fact that it was not always possible to demonstrate their presence in many known cases of gonorrhea, and because the fixation of complement was slight when it did occur, this has been a difficult question to answer.

Complement fixing bodies are present in gonorrheal serums, but they are present in such small amounts that the limitations placed on the test by the customary technic must be disregarded in order to detect them.

In the Wassermann test when the total volume is 0.5 c.c., the difference between the average four-plus serum and the average control tube is at least 0.05 c.c. of complement, and it may be considerably more. The conditions surrounding the gonorrhea test are quite different. Many known positive serums cannot be made to show anything but a plus-minus result. In this case, the

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difference between the amount of complement fixed in a positive test tube and the amount absorbed by the control tube is very small.

In the classical gonorrhea complement fixation test the total amount of complement fixed is small even under the best conditions, therefore we are deprived of the latitude which exists in the Wassermann test whereby the positives can be graded between four plus and negative. In gonococcus fixations, the total complement fixation for the average positive serums is seldom in excess of 0.02 c.c. of complement. This leaves little room for graduation of the positives except in occasional cases. The gonorrhea test therefore resolves itself into a problem necessitating the detection and quantitative estimation of exceedingly small amounts of complement.

Small differences in complement strength cannot be estimated accurately if the concentration of complement present is large. When large concentrations of complement are used in gonorrhea fixations, the large quantities left over make it impossible to detect the small amounts that have been fixed. For accurate quantitative determinations of complement, its concentration must approach the threshold value as indicated in our paper on "The Inadequacy of Present Complement Titrations".<sup>2</sup> Therefore, in a proper gonorrhea fixation test, it is imperative that the readings be made at the point where the concentration of complement approaches its threshold value.

However, when the concentration of complement is reduced, the test can not be completed in the usual manner because the control tubes will be anti-complementary and the reaction tubes will not show definite positive or negative reactions. In addition, most positive gonorrhea serums do not completely fix the complement even though it is present in small amounts.<sup>3</sup> As a result, these positive specimens show a trace of hemolysis with the ordinary 5 per cent cell suspensions. This hemolysis disguises the true positives.

A properly planned titration demonstrates how these difficulties may be overcome. To a series of tubes, each containing an equal amount of complement, are added graded amounts of sensitized cells. The volumes of the reacting solutions are equalized with normal saline. The complement is of such strength that only the tube having least cells is completely hemolyzed. From this it is seen that a small amount of complement can completely hemolyze a cell suspension when the cell density is reduced. Furthermore the supernatant fluids of the succeeding tubes show that although a given amount of complement may be insufficient to hemolyze completely a light cell suspension it will produce more hemolysis when it reacts with cell suspensions of greater density.

Therefore when the first requisite of gonorrhea fixation tests is fulfilled, i.e. the use of reduced concentration of complement, to get clearer control tubes and clear cut negative reactions, the cell suspensions must be lighter than those used in present work. Furthermore, as the density of the suspensions is decreased, hemolysis disappears in the positive reaction tubes, and definite positive reactions are at last attained.

We have found that when one fifth of the reacting complement fixation solution is made up of sensitized cells, highly satisfactory results are obtained. The sensitized cell suspension consists of one part of 5 per cent cells and one and one half parts of hemolysin of the proper titer.

It has been demonstrated that unless the amounts of nonspecific complement absorption of any and every serum are taken into consideration, the results are not of much value in truly quantitative fixation determinations.<sup>2</sup> The standard that determines our readings is that complement fixation should be judged by the difference between the amount of complement absorbed nonspecifically in the control tube and the amounts fixed both nonspecifically and specifically in the reaction tubes.

When a series of duplicate complement fixation tests are run with the same positive specimen, each test differing from the other in the amounts of serum used, it will be seen that fixation is not directly proportional to the amounts of serum present. In other words, when one tube has twice the serum of the second, the first tube does not necessarily have twice the fixation of the second. Also, when the fixations of weakly positive serums are compared with one another, it is seen that doubling the serums in one case may double the fixation, while with another doubling the amount of serum may not increase the fixation at all.

An essential difference between the proposed test and the classical is that we do not attempt a quantitative estimation of complement fixation by varying the amount of serum, but by changing the concentration of the complement in the hemolytic system, and by estimating the amount of fixation by the threshold value of the complement left over. In this new test we take advantage of the fact that in hemolytic systems, complement acts according to its concentration rather than its absolute quantity.<sup>2</sup>

#### THE LABORATORY SET-UP FOR THE TEST

TABLE I

THE COMPLEMENT TITRATION  
INCUBATION AT 37° C. FOR ONE-HALF HOUR

Pooled serum	0.05	0.05	0.05	0.05	0.05	0.05
Complement	0.05	0.06	0.07	0.08	0.09	0.10
Normal saline	0.05	0.04	0.03	0.02	0.01	0.00
Normal saline	0.82	0.82	0.82	0.82	0.82	0.82
Sen. cells	0.23	0.23	0.23	0.23	0.23	0.23

That quantity of complement to be used in the tests is the amount which causes almost complete hemolysis. The proper tube will have a trace of cloudiness caused by cells that have not been hemolyzed. The complement is then diluted so that 0.1 c.c. contains that amount indicated by the titration.

TABLE II

THE COMPLEMENT FIXATION TEST FOR GONORRHEA  
INCUBATION AT 37° C. FOR ONE-HALF HOUR

	CONTROL	I	II	III
Serum	0.05	0.05	0.05	0.05
Complement	0.1	0.1	0.1	0.1
Antigen	0.0	0.1	0.1	0.1
Normal saline	0.82	0.6	0.4	0.2
Sen. cells	0.23	0.2	0.15	0.1
Total volume	1.15	1.0	0.75	0.5

It is essential that the amount of antigen used is an excess. Additional antigen should not increase the amount of fixation of the positive specimens.

For ease of manipulation, following the first incubation, the saline and sensitized cell suspension should be mixed together and added by a single pipetting and the specimens reincubated. This also makes for increased accuracy.

#### INTERPRETATION OF RESULTS

The tests are read after the cells have settled to the bottom of the tubes. A test can be read only when the control tube is almost completely hemolyzed. When it is completely hemolyzed, too much complement has been used, and the test has to be repeated. When the control has much more than a trace of cells left over, the test cannot be read for the positive cases. These cases will have to be repeated. When the amount of complement for the serums is well chosen, all the tests can be read except those which are markedly anticomplementary.

- Reaction The first reaction tube is as much hemolyzed as the control.
- + Reaction The first reaction tube colorimetrically shows definite fixation against the control. The second reaction tube approaches complete hemolysis.
- 2+ Reaction The first reaction tube has only a faint trace of hemolysis or is completely fixed. The second reaction tube has much hemolysis.
- 3+ Reaction The first reaction tube is completely fixed. The second reaction tube has a faint trace of hemolysis or is completely fixed. The third reaction tube has definite hemolysis.
- 4+ Reaction All the reaction tubes are completely fixed.

The test becomes valueless when the antigen is anticomplementary. It is satisfactory if after a fixation with a negative serum, the first reaction tube is as much hemolyzed as the control. Serums tend to reduce the anticomplementary properties of the antigen, and for that reason, to ascertain whether an antigen is suitable from this standpoint, fixations must be run with negative serums.

The effects of cell fragility are minimized in this test because the factors that affect the control, affect the reaction tubes identically. Since the reaction tubes are read against the control, it makes no difference whether the cells are resistant or fragile to the action of complement.

The test is very delicate and errors in technique show up immediately. When the complement is of too high a concentration, the control tubes are completely hemolyzed, and when it is of too weak a concentration, the control tubes have too little hemolysis to permit reading the fixation of the positive specimens.

#### SUMMARY AND CONCLUSION

We believe that we have developed a sensitive, truly quantitative, and stable method for estimation of complement fixation.

The test is sensitive because with a reduction in the concentration of complement used, clear cut definite positive and negative reactions have been attained.

It is quantitative because the amount of nonspecific complement absorption for every specimen is accurately calibrated and only the specific fixation is read,

and because equal quantities of serum are used in the control and in each of the reaction tubes.

Finally more uniform results are obtained by this method since the effects of cell resistance to complement action are minimized; and also since the effects of variation in cell suspension density are minimized, due to the reading of the reactions at the threshold values of the complement.

The principles underlying this test are applicable to other fixations.

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### THE INADEQUACY OF PRESENT COMPLEMENT TITRATIONS\*

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THE unit of complement is defined as the smallest amount of complement which will completely hemolyze 0.1 c.c. of 5 per cent sheep cells in the presence of an excess of hemolysin after an hour's incubation at 37° C. Despite the clarity of the definition, this hemolytic unit of complement is an indefinite and variable quantity. Complement fixation determinations which depend upon it for their accuracy are similarly affected. The reason for this is that the several factors involved in the determination of such a unit are variable and beyond laboratory control.

For example, the density of the cell suspension decides the size of this unit, for when the suspension is heavy, the unit is larger than when the suspension is light. Yet it is difficult if not impossible to make up suspensions of constant density. Efforts<sup>1, 2</sup> to produce a standard 5 per cent cell suspension based on colorimetric nephelometry and cell counts have been abandoned when experiments have shown them faulty.

Even if standard suspensions were achieved, the problem would not be solved because it is not possible to pipette absolutely uniform amounts of cells into a series of tubes from any suspension. In addition to the technical difficulties of pipetting equal volumes of fluid, there is the factor of the cells constantly settling out in the stock solution and even in the pipette. This makes standard cell suspensions theoretical rather than actual. Therefore, all methods of estimating complement strength and all complement fixations which require standard suspensions are subject to variation. In order to achieve accuracy, complement titrations and fixations must be independent of the inevitable variations in cell suspension density.

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Another obstacle to establishment of a standard complement unit is the difficulty of determining the point of complete hemolysis. Brooks<sup>1</sup> recognized this and proposed the use of the point of 50 per cent hemolysis as a criterion of the amount of complement present. Wadsworth, Maltaner and Maltaner<sup>2</sup> state, "The titration of complement to the point of complete hemolysis for the unit value, as compared with titration to the point of 50 per cent hemolysis, proved so inaccurate as to obscure completely the true quantitative relations that exist in the course of the reaction." Although they found that reading from 50 per cent hemolysis is much more accurate than reading from the point of complete hemolysis, nevertheless this advancement is not sufficient to bring the test to the degree of accuracy required by this type of work.

While this method avoids the necessity of determining the point of complete hemolysis, it is not free from the objection cited above, namely, the impossibility of preparing and pipetting a standard cell suspension.

Any such method contains other and equally serious faults. In hemolytic titrations when there is sufficient complement to produce approximately 50 per cent hemolysis the addition of relatively large variations in the amount of complement produce small changes colorimetrically. Moreover, the eye is unreliable when called upon to distinguish or estimate these differences in the amounts of hemolysis even when they are considerable. The amounts of complement which produce these undetectable changes in the degrees of hemolysis are nevertheless sufficient to produce wide variation in the results of complement fixation tests. In other words, this type of titration is not sufficiently sensitive and is of limited value in keeping complement fixation results accurate or stable.

The following work leads to a better estimation of complement strength.

When supernatant fluids of a complement titration are examined in a colorimeter, it is found that the amount of hemolysis obtained is not directly proportional to the amount of complement present. When complement is of such a concentration that 0.1 cc produces a slight amount of hemolysis 0.2 cc produces more than twice that amount. The stronger the complement the smaller are the differences in hemolysis caused by 0.2 cc and 0.1 cc complement, and conversely, the weaker the complement, the greater are these differences. Therefore, by looking at one tube of a titration it is impossible to predict what will happen in any other tube.

In an experiment of this kind when the hemolytic solutions were examined in a colorimeter, the solution containing 0.2 cc of complement had four times as much hemolysis as the tube containing 0.1 cc of complement. Doubling the amount of complement increased the color of the hemolytic solution four times. Since the addition of the second tenth of a centimeter of complement produced three times the amount of hemolysis as the first tenth, apparently only one third or 0.033 cc of the first 0.1 cc of complement produced any color, and 0.067 cc of it was used up in overcoming the resistance of the cells. This was shown true when a tube containing 0.067 cc of complement failed to produce hemolysis. Applying the same reasoning to any hemolytic system, one concludes that only part of the complement present produces the hemolysis. The amount of complement used up in overcoming the resistance of the cells to



hemolysis plays an important part in estimating complement strength. In this paper, this amount of complement will be referred to as the threshold value of the complement and is represented in the titration by the largest amount of complement which may be added to the hemolytic system without producing any hemolysis.

Choosing any two tubes of a titration and applying to them the equation listed below, the result will always be the threshold value of the complement.

$$A - \frac{B - A}{\frac{X}{Y}} = \text{the threshold complement value}$$

A smaller amount of complement

B larger amount of complement

X colorimetric reading for the solution containing the smaller amount of complement

Y colorimetric reading for the solution containing the larger amount of complement

Like standard cell suspensions, this formula is theoretical rather than actual. It is applicable for tubes that have no more than 50 per cent or less than 10 per cent hemolysis. The factor which causes this limitation and also adversely affects the formula is that as cells hemolyze because of the action of the complement, the resultant hemolysis inhibits further action of the complement. This will be discussed shortly. The formula is also affected by the fact that no two tubes contain the same amounts of cells, and by the technical difficulties of pipetting exact amounts of complement, and by our ability to read colorimeters, and by the difficulty of judging where hemolysis begins. Because of all this, the figure obtained by the equation, instead of checking perfectly, may approximate the largest amount of that complement that may be added to the hemolytic system without producing any hemolysis.

The fact that any two tubes of a titration when resolved according to the above equation always point to the threshold tube—i.e., the tube containing the most complement which has no hemolysis—makes this tube the most stable in any titration. In actual practice it is easier to find the threshold value than to predict it.

It is for several reasons that the tube which contains the least amount of complement above the threshold value, i.e., the first tube that shows hemolysis, is the most important in any titration. In this tube small changes in the amounts of complement produce easily detectable variations in the amounts of hemolysis. Amounts of complement that produce no visible changes when hemolysis is 50 per cent or more, produce startling changes where hemolysis begins. Another justification for the reading of complement titrations from this tube is that small changes in the density of the cell suspension which would markedly affect the point of 50 per cent hemolysis, have almost no effect on the tube which shows little hemolysis. Therefore, by using the least hemolyzed tube of a titration to estimate complement strength there are two positive advantages to be gained; one is that small changes in complement strength are easily detected and the other is that the effects of cell density variation are minimized.

Aside from these advantages the following experiment reveals additional considerations for this type of titration reading. Taking account of the fact that complement acts according to concentration and not to absolute quantity:

a titration may be made with constant amounts of complement and sensitized cells but with increasing amounts of salt solution. This kind of titration shows that it is possible to make any quantity of complement produce any amount of hemolysis by simply changing the volume of solution in which it is allowed to act. And so it is possible to obtain more hemolysis with 0.1 cc. of complement in a small reacting volume than with 0.2 cc. in a volume so large that its concentration is less than that of the 0.1 cc. This important fact has hitherto not been used to its fullest advantage in complement fixations.

We slightly digress to note that in systems consisting of patient's serum, bacteriolytic antigen, and complement, the complement acts according to the absolute quantity present rather than according to its concentration. Of a given amount of complement, the same quantity is fixed specifically by the immune substances whether the reacting volume is large or small. We have found this to hold true in Wassermann and gonorrhea fixations. This definitely establishes the fact that bacteriolytic systems are different from hemolytic.

We can demonstrate that as hemolysis proceeds in any tube, the internal osmotic pressure of the cells falls because of the loss of electrolytes from the cells. As the process goes on, the electrolytes lost by the cells are added to the outside solution which is thereby raised in osmotic pressure until the inside and outside pressures of the cells are in equilibrium. The more hemolysis there is in any tube, the greater is the resistance effort of the suspension against further action of the complement. Any tube that shows considerable hemolysis does not represent the absolute potency of the complement present.

From this it is seen that it is a mistake to assume that because graded amounts of hemolysis are obtained with graded amounts of complement hemolysis ceases when the complement is used up by the hemolytic system. In every tube of a titration when hemolysis stops, there is still some free complement, but its concentration is too low to produce any more hemolysis.

Returning to the estimation of the value of a faintly hemolyzed tube, it will be seen that in addition to the advantages listed above, it also has the advantage of not being affected by the back pressure produced by a solution containing hemoglobin and expelled electrolytes. In fact, it is the only tube in a titration which can be depended upon to give a true indication as to the strength of the complement.

A final consideration is that complement titrations, no matter how made or evaluated, are influenced by cell resistance to complement action. In any titration, it is difficult to determine to what degree the amount of hemolysis obtained is influenced by this fact. The same picture is obtained when the complement is strong and the cells resistant as when the complement is weak and the cells fragile. This is perhaps the most disturbing factor encountered in complement fixation work and the results of all complement fixation reactions are influenced when cells of the two extremes are used. When complement is titrated for its hemolytic activity without regard to its bacteriofixing value, as is commonly done and when different lots of cells are used on successive fixations with a positive specimen different values will be obtained for specific complement fixation. When the cells are weakly resistant to hemolysis, more

immune substances will be credited to the specimen than when the cells are strongly resistant to hemolysis.

The effects of variation of cell resistance to hemolysis can be minimized by a titration of complement against a standard border-line four-plus serum. A second titration should be run to determine the density of the cell suspension that would give complete hemolysis with negative specimens.

This sort of titration would tend to keep results uniform, but like all other present titrations, fails to take into account anticomplementary substances any specimen may contain. When, regardless of the age of the serums, sufficient anticomplementary substances are present so that 0.08 c.c. of any serum is capable of absorbing from 5 per cent to 100 per cent or more of that complement necessary to hemolyze completely 0.1 c.c. of a 5 per cent cell suspension, titrations are not of much value in truly quantitative fixation determinations unless they indicate the amounts of complement nonspecifically absorbed by each and every specimen.

Complement fixation by serum and antigen above the amounts absorbed by the anticomplementary substances is specific complement fixation when the antigens are not anticomplementary. When the sole difference between the reaction tube and the control tube is the lack of antigen, and when hemolysis approaches completion in the control tube, any lack of hemolysis in the reaction tubes is due to specific fixation. The quantitative determination of this is important and will be discussed in a later paper.<sup>2</sup>

#### SUMMARY AND CONCLUSIONS

Titration and fixations must be read where hemolysis begins, so that they will be independent of the inevitable variations in cell suspension density; and so that they will be sensitive—for amounts of complement that produce no changes where hemolysis is appreciable, produce detectable changes where hemolysis begins; and finally, so that the resistance to further complement action, engendered by hemolysis, will be minimized.

Furthermore, systems that do not take into account the amounts of non-specific complement absorption of every specimen, fail to be quantitative.

We have taken advantage of the material briefly outlined in this paper, and as a result, think we have succeeded in developing more sensitive fixation tests.<sup>2</sup>

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## A METABOLISM CHAMBER WHICH AUTOMATICALLY MAINTAINS A CONSTANT PARTIAL PRESSURE OF OXYGEN\*

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IN 1850 Regnault and Reiset<sup>1</sup> described an apparatus for measuring the respiratory exchange of animals, consisting of a closed chamber in which the animal was placed, a system for circulating the air and removing the carbon dioxide and water vapor excreted, and a means for automatically supplying oxygen to the chamber to replace that absorbed by the animal. The principle of this so-called 'closed circuit apparatus' has been employed in numerous instruments devised for the study of respiration, metabolism, and anoxemia, notably the calorimeters of Atwater and Benedict and of Lusk, the clinical metabolimeters of Krogh and of Benedict, and the rebreather used in the examination of government aviation personnel.

In the usual laboratory form of this apparatus the air of the animal chamber is circulated continuously by a pump, passing through soda lime which absorbs the carbon dioxide, and either sulphuric acid or an ice chamber, which removes the water vapor. The subtraction of these gases decreases the pressure in the chamber, this causes an equivalent volume of oxygen to flow in from a gasometer which is at atmospheric pressure, thus restoring the chamber pressure to normal. However, this closed system of chamber and absorbers is in effect a combined gas thermometer and gas barometer, a rise in temperature or a drop in barometric pressure causing increased pressure within the system, and vice versa. Since the only communication between the system and the atmosphere is through the gasometer, such pressure changes must interfere greatly with the normal flow of oxygen into the system, causing correspondingly large variations in partial pressure of the oxygen in the animal chamber. For example, the combined effect of the extreme but possible atmospheric changes of +20° C and -40 mm Bar is to increase the pressure or volume of a gas 10 per cent. This is of little importance in experiments where the partial pressure of oxygen in the animal chamber is 300 to 500 mm Hg, but in studies requiring an oxygen tension of 75 mm Hg or less, changes of such magnitude might double the tension or reduce it to zero. Hence, frequent gas analysis and constant adjustment of the oxygen supply are necessary in order to maintain the oxygen tension at the desired level.

The present apparatus was designed to compensate for temperature and pressure effects, and to eliminate the necessity for constant supervision. It is shown diagrammatically in Fig 1. Chamber A is occupied by the animal. Air circulates from the top of this chamber, through the carbon dioxide absorbers D, the motor driven pump P, the ice chamber (water remover) G, and back

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to the bottom of the chamber. On either side of the animal chamber are the compensating chambers *B* and *B'*, which are closed to the atmosphere and the animal chamber but are open to the tank *C*. By running water into this tank

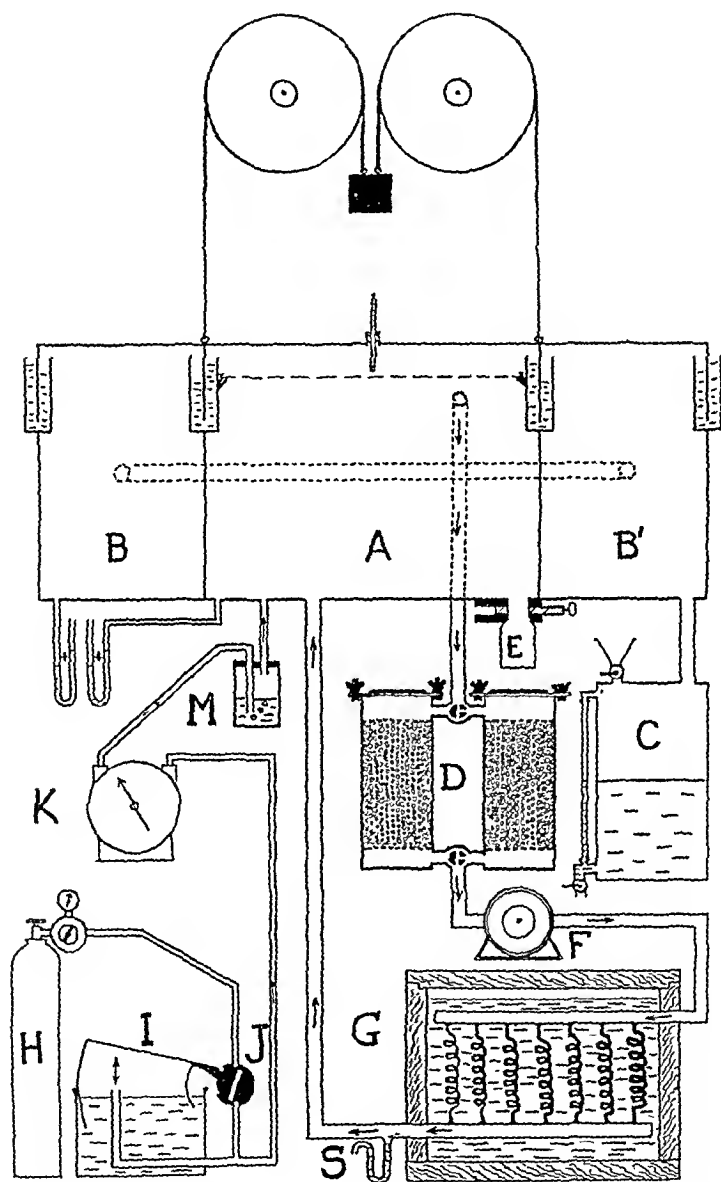


Fig. 1.—*A*, animal chamber. *B* and *B'*, compensating chambers. *C*, tank for adjusting air volume of compensating system. *D*, carbon dioxide absorbers. *E*, glass jar attached to sliding valve, for collecting excreta. *F*, air circulating pump. *G*, ice chamber for condensing water vapor. *S*, syphon trap. *H*, oxygen cylinder with reducing valve. *I*, oxygen gasometer. *J*, automatic oxygen valve. *K*, oxygen meter. *M*, Mueller valve.

the gas volume of the compensating system may be adjusted to equal that of the animal chamber and absorbing system. The cover, common to the animal chamber and the compensating chambers, is carefully counterbalanced and

moves freely up and down like the bell of a gasometer. The area of the cover of *A* equals the combined areas of the covers of *B* and *B'*. Both systems have valves opening to the atmosphere for use in adjusting the chamber pressures, and each system is provided with a water manometer indicating the chamber pressure relative to that of the atmosphere.

At the start of an experiment the animal is placed in chamber *A* and the cover lowered until its side walls are half submerged in the water seals. The valves to the atmosphere are then closed, the pump started, and by manipulating one of the valves air is withdrawn from the animal chamber until its manometer reads  $-10$  mm. Since the two chamber systems contain equal volumes of gas and have covers of equal area, the mechanical effect of the minus pressure, transmitted through the cover to the gas in the compensating system, causes the manometer of chamber *B* to read  $+10$  mm. The Mueller valve *M* has a resistance of about 10 mm of water, and as the animal's absorption of oxygen increases the negative pressure in *A* this resistance is overcome and oxygen flows into the system from the gasometer *I*, through the oxygen meter *K*. In the usual apparatus any change in room temperature or barometric pressure interferes with the normal inflow of oxygen. In the present apparatus, however, the gas in the compensating system expands or contracts equally with that in the animal chamber system, the cover rises or falls to compensate for the change in volumes, and the pressure relations remain unaltered. Hence the negative pressure in the animal chamber varies little, oxygen flows in according to the needs of the animal, and the oxygen tension is maintained within narrow limits.

Theoretically the variation in oxygen tension in the chamber should be no greater than that of the atmosphere. Practically it is somewhat larger, owing to the friction of moving parts of the apparatus and the difficulty of eliminating small leaks. Nevertheless, a series of three day runs during periods of very changeable weather indicates that the oxygen tension in the chamber may be kept within limits of  $\pm 3$  per cent.

*Details of Construction and Operation*—The chamber and cover are made of 18 gauge galvanized iron, carefully soldered at the seams. The animal chamber is 90 cm long, 75 cm wide, and 70 cm deep, and has a volume of about 500 liters. The compensating chambers have similar vertical and lateral measurements but are only one half as long, making their combined volume equal to that of the animal chamber. The water seals are 20 cm deep, and the side walls of the cover are 22 cm high. The effective volume of each cell of the cover is approximately one fifth of that of the corresponding chamber. The cover and counterweight are suspended on 18 gauge piano wire from a pair of bicycle wheels mounted on the ceiling. Change of cover weight due to its immersion in the water seals was originally compensated for by the use of a mercury syphon attached to the counterweight but this refinement was found unnecessary in practice. A vertical scale on the cover, calibrated in terms of volume, is used when making gas mixtures in the chamber.

Oil-impregnated maple set in petroleum pitch covers half of the animal chamber floor, and provides a comfortable resting place for the animal. There

is a large plate-glass window in the front of the chamber, and a smaller one in the cover. At the top of the animal chamber is a grill (Fig. 1) which prevents the animal from striking its head against the cover. All connections between the chambers, tank, and absorbers are made with standard one-inch iron pipe, well painted at the joints to prevent leaks.

Since the animal usually remains in the chamber for several days, provision has been made for removing excreta and supplying food and water without raising the cover or otherwise changing the oxygen tension appreciably. In one corner of the chamber floor, there is an opening 7.5 cm. in diameter, fitted with a sliding gate valve. The gate is made of 3 mm. German silver, the rubbing surfaces are ground to a careful fit, and uniform pressure between parts is maintained by means of springs. This type of valve is not gas-tight, but in the present situation the valve is always wet and moisture drawn into the crevices by capillary action provides a perfect seal for the pressures in use. The bottom of the valve is threaded to receive a screw top glass jar (*E*, Fig. 1), which seats tightly against a soft rubber gasket. Immediately above the valve opening, the side walls of the animal chamber have been cut away

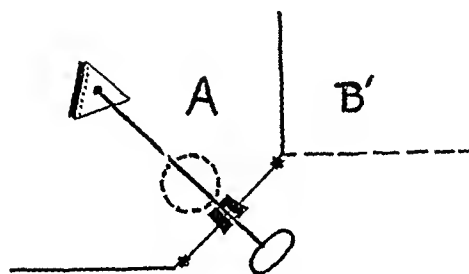


Fig. 2.—Plan of rubber window and sliding rake, for cleaning animal chamber.

(Fig. 2) to permit the placing of a round window 15 cm. in diameter, set perpendicular to the floor and at an angle of  $45^\circ$  to the long dimension of the chamber. The adjacent corner of the compensating chamber is also cut away to accommodate this construction. The window is made of soft sheet rubber 5 mm. thick, and in its center a large rubber stopper is fixed. The stopper is pierced with a 6 mm. hole through which passes the metal handle of a rake. The handle is lubricated with petrolatum and slides easily through the stopper without causing leaks. The blade of the rake is a triangle of steel, and along one edge is fixed a strip of rubber. The sharp edges of the rake dislodge adherent matter, and the soft rubber edge is used to shift semiliquid material. Since the window is set at an angle of  $45^\circ$ , it is possible to reach any part of the chamber floor, and to rake solid excreta and débris to the valve opening where it falls into the glass jar. When the jar is full, the valve is closed and the jar is removed and replaced by another. Ordinarily the valve remains open, and since the floor slopes from all directions toward the valve opening, urine drains into the jar as soon as it is deposited.

Food is introduced into the chamber by the mechanism shown in Fig. 3. The food carrier is a metal cylinder 18 cm. long and 7.5 cm. in diameter, closed at the ends, fitted with a leather compression ring near each extremity, and

attached to a handle. It is open at one side to receive the food. Lubricated with petrolatum, it slides in the tightly fitting outer tube which passes through the side wall of the chamber. When the carrier is at the outer end of its stroke, ready to receive food, the seal is maintained by the inner compression ring, when it is at the inner end of the stroke the greater part of the carrier is within the chamber and the seal is maintained by the outer compression ring. In this position, the carrier is rotated 180° by means of the handle, and the food drops on the chamber floor. Water is automatically supplied to a drinking trough through a siphon arrangement.

The carbon dioxide absorbers (*D*, Fig 1) are metal cylinders 22 cm in diameter and 45 cm high, fitted with covers sealed by rubber gaskets. Each holds about 20 pounds of 48 mesh soda lime. A screen at the top serves to trap any hair or other matter which may be drawn in from the animal chamber. On the bottom screen is a thin layer of cotton which prevents lime dust from leaving the absorber. A pair of three way valves worked simultaneously by the same lever permits one absorber to be refilled while the other is in operation.

The pump (*P*, Fig 1) is an eccentric type positive blower, directly con-

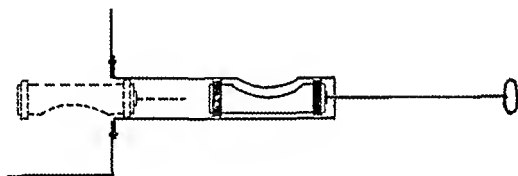


Fig 2—Elevation of food carrier mechanism

needed to a variable speed  $\frac{1}{2}$  H P electric motor running at a speed of 450 r p m. The ventilation rate of 500 liters per minute keeps the carbon dioxide and humidity within normal limits.

The ice chamber or water remover (*G*, Fig 1) is a metal box 60 cm long, 45 cm wide, and 75 cm deep, insulated with a 7.5 cm layer of sheet cork on all sides, and containing 6 vertical coils each consisting of 15 meters of 1 cm copper tubing. These coils are connected to the inflow and outflow pipes at top and bottom. The chamber is filled with ice and ice water, and as the circulating air passes through the coils, water is condensed out of it and escapes to the drain through the siphon *S*. The outflow pipe is insulated with cork, and the air returns to the animal chamber cool and comparatively dry.

The Mueller valve (*M*, Fig 1) is of the usual type. The depth to which the tube is immersed in the water determines the negative pressure in the animal chamber, therefore the tube should have an internal diameter of at least 1 cm, and V shaped notches filed in its walls at the tip so that the oxygen may escape into the water in small bubbles.

The oxygen meter (*K*, Fig 1) is the usual rotary wet meter, it should be kept filled to the mark with water, and calibrated periodically against a standard gas holder.



The gasometer (*I*, Fig. 1) is of the segment type; the bell is 10 cm. wide and 45 cm. long, is counterbalanced, and moves freely on ball bearings. Its shaft operates valve *J*, thus controlling the flow of gas from the reducing valve of the oxygen cylinder *H*. As oxygen is drawn from the gasometer by the negative pressure in the animal chamber the bell descends, opening valve *J* and allowing oxygen from the cylinder to flow into the system. Any excess over the amount required in the animal chamber passes into the gasometer bell, causing it to rise, thereby closing valve *J* and cutting off the flow of oxygen from the cylinder. The gasometer is, in effect, a second reducing valve, simple and very sensitive, and this arrangement supplies oxygen automatically until the cylinder is empty.

This apparatus was designed for the rather special purpose of studying animals under conditions of lowered oxygen tension, and the metabolism was calculated on the basis of the oxygen consumption, without determining the respiratory quotient. More precise results would doubtless be obtained if the wet meter were kept in a constant-temperature box; and the respiratory quotient could be determined by substituting the usual sulphuric acid-soda-lime-sulphuric acid train for the present carbon dioxide absorbers and ice chamber. If this system were adopted, the water-seals of the cover should be filled with mineral oil.

#### SUMMARY

A new apparatus for the study of gaseous metabolism is presented, incorporating the closed circuit principle of Regnault and Reiset, with means of automatically compensating for the effects of temperature and barometric pressure on the oxygen tension in the animal chamber. Mechanisms for supplying food and removing excreta without appreciably altering the oxygen tension, and of automatically delivering oxygen from a high pressure cylinder to the animal chamber, are described and illustrated. The oxygen tension is maintained within  $\pm 3$  per cent, as checked by frequent gas analysis, and an animal may live comfortably in the chamber for several weeks.

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# DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

## HEMOPHILIA, *Histopathology of Hematopoietic Tissues in*, Custer, R. P., and Krumbhaar, E. B. *Am J M Sc* 189 620, 1935

Three fatal cases of hemophilia with necropsy are reported, 1 patient dying from an uncomplicated hemorrhage, 1 from appendicitis and hemorrhage, and 1 from fulminating pneumonia.

The hematopoietic tissues all showed normal regenerative ability, the first two predominantly erythroblastic, the third leucoblastic.

All three showed a marked increase of megakaryoblasts and megakaryocytes in the bone marrows, indicating a relationship of the blood platelets to the hemophilic process.

J. H. Wright's observation of platelet formation in the bone marrow sinusoids from intruding pseudopods of megakaryocyte cytoplasm was supported by the findings.

## EOSINOPHILIA, in Scarlet Fever, Friedman, S. *Am J Dis Child* 49 933, 1935

An eosinophil count was made in 100 cases of scarlet fever in the early stages of the disease. In 46 per cent of the cases eosinophilia was demonstrated in a single count.

Eosinophilia is most likely to be present in the early stages of the disease in the mild cases in which there are mild constitutional symptoms and a mild rash. The incidence of eosinophilia in such cases was from 60 to 70 per cent as compared with 46 per cent in all cases of the disease. It is in the mild type of case that frequently there is difficulty in making the diagnosis. The use of the presence of an elevated eosinophil count as an aid to diagnosis is recommended.

## PNEUMONIA, Incidence of Bacteremia in and Its Relation to Mortality, Bullowa, J. G. M., and Wilcox, C. *Arch Int Med* 55 558, 1935

The incidence of bacteremia in connection with the fatality rate is listed for different types of pneumococcal pneumonia in a series of 1,725 cases due to a single type, occurring over a five year period, at Harlem Hospital.

The percentage of invasion of the blood differs for the different types, though in the main the percentages of invasiveness and mortality are approximately the same.

The importance of (1) differentiating the types formerly included as group IV or the x group and (2) of typing individual cases for prognosis and eventual treatment, when specific treatment is available, is manifest.

Only 3 per cent of pneumococcal pneumonias escape classification at the present, when typing is carried from I to XVIII (Cooper).

The variation in percentage of invasion and fatality from year to year in different pneumococcal types is evidence of the variations which are encountered from season to season, and shows the necessity of extending over several years the testing of any proposed curative substance.

## INFECTIONS, by Gas Forming Anaerobic Bacilli, Reeves, J. R. *J A M A* 104 526, 1935

Approximately 30 per cent of cases reported and treated as *Clostridium welchii* gas gangrene are caused by other anaerobic organisms.

This 30 per cent of cases should be classed as putrefactive gangrene and treated conservatively by systemic supportive treatment, débridement and irrigations. *Clostridium welchii* antitoxin is not indicated and may be injurious.

Putrefactive gangrene is likely to appear in patients past the age of fifty years who are constitutionally below normal. Cases appear notably in patients with circulatory failure, arteriosclerosis, thromboangiitis obliterans and diabetes mellitus.

The direct cause for putrefactive gaseous lesions in the muscles of patients whose condition has been described may be bruises, burns, simple fractures, parenteral administration of drugs and solutions, and circulatory failure.

Putrefactive gas-forming anaerobic soil bacteria contaminate food at all times. It seems apparent that bacteria of this type are frequently present in the organs and muscles of the aged individual.

Diagnosis of *Clostridium welchii* infection cannot be based on direct smear; the rabbit inoculation test or identification is best corroborated by the use of anaerobic cultural methods and related criteria.

Patients suffering from wounds, particularly of muscle tissue, which have been contaminated with soil or street dirt, should be given gas gangrene antitoxin prophylactically and in addition should receive débridement and irrigations. If toxic symptoms develop radical débridement, continuous irrigations, and therapeutic serum are indicated without delay.

**TULAREMIA**, Kavanaugh, C. N. Arch. Int. Med. 55: 61, 1935.

In addition to a short review of the literature, the characteristic clinical features in 123 cases of tularemia are presented, together with observations at autopsy in one case.

A new classification is presented. Sixty-nine cases occurred in males and 54 in females. One hundred and eight were of the primary cutaneous type, 6 were of the primary ophthalmic type and 9 were of the cryptogenetic type of the disease.

The average incubation period, definitely determined in 58 cases, was four and one-half days.

Sixteen cases presented pulmonary involvement. Primary and secondary pulmonic tularemia are discussed. The breast was involved in 2 cases. Thrombi in the veins were noted in 1 case. Pleurisy was a complication in 6 cases. Abdominal symptoms were prominent in 5 cases. Peritonitis was present in 1 instance. Delirium was a prominent feature in 6 cases. Acute mania complicated 1 case. In 1 case of the primary ophthalmic type optic atrophy occurred, with loss of vision in the affected eye. A purulent dacryocystitis complicated 1 case. Decided splenomegaly with perisplenitis was present in 5 instances. Osteomyelitis was observed once. Cervical adenitis as the only external evidence of the disease was observed in 4 cases. Subcutaneous nodules occurred in 28 instances. A cutaneous eruption was present in 23 cases (18.7 per cent).

There were 5 deaths, indicating a mortality of 4 per cent in this series.

Other pertinent facts pertaining to the history, epidemiology, pathology, diagnosis, and treatment are also discussed.

**RETICULOCYTES**, Fragility and Maturation of, Mermod, C., and Dock, W. Arch. Int. Med. 55: 52, 1935.

The reticulocytes, and particularly the young (heavily stained) ones, are more easily destroyed in shed blood and in the circulating whole blood by saponin (and by citrate and oxalate in whole shed blood) than are the other red cells.

The maturation of reticulocytes in the circulating blood is not proved; it seems not to occur on any significant scale.

The occurrence of a reticulocytic crisis early in the treatment of anemia is probably an unavoidable evil associated with a change in the structure and function of the bone marrow. This precedes and delays the rise in the red cell count.

**ENCEPHALOPATHY, Lead, Simulation of Intracranial Tumor, Bucy, P. C., and Buchanan, D. N. J. A. M. A. 105: 244, 1935**

In two of three cases of lead encephalopathy in children with increased intracranial tension simulating cases of intracranial tumor, a suboccipital decompression was made. Both children recovered promptly and completely. In the third, in which the intracranial pressure was less elevated, no decompression was made and medical management alone was used. This child had a prolonged and stormy course but also ultimately recovered. From these experiences and from data taken from the literature, it is concluded that:

1. The differentiation of lead encephalopathy and midline cerebellar tumors in children from the history and physical signs alone is difficult and may be impossible.

2. Such factors as the possible ingestion of lead, rapid onset with vomiting and abdominal pain, and only moderately increased intracranial pressure should warn the clinician of the possible diagnosis of lead encephalopathy.

3. The easiest and most rapid method of rendering probable the diagnosis of lead encephalopathy is the demonstration of the presence of lines of increased density at the ends of the long bones. The diagnosis may then be established by demonstrating the presence of lead in the blood.

4. Medical management of lead encephalopathy is notoriously unsatisfactory and results in a high mortality and numerous and serious sequelae.

5. Surgical decompression of the intracranial cavity offers a logical means of producing a prompt and complete relief of symptoms with less danger of sequelae.

**TYPHOID VACCINE, Study of Agglutinin Response to, Valentine, E., Park, W. H., Folk, G., and McGuire, G. Am. J. Hyg. 22: 44, 1935**

The rise in the agglutinin titers of the blood serums of rabbits and human beings, following the percutaneous administration of typhoid vaccine, paralleled the number of treatments and the size of the dose. The human beings gave small rises in agglutinin titer after a large number of rubbings, the rabbits with a greater number of rubbings than the human beings, larger doses, and a relatively greater rubbing area, gave good agglutinin titers.

Intracutaneous injections of typhoid vaccine in weekly doses of 50, 100, and 150 million bacilli produced as great an agglutinin response as subcutaneous injections of 100, 200, and 300 million, or 250, 500, and 1,000 million bacilli. Intracutaneous injections of 25, 50, and 100 million produced slightly less response.

No appreciable systemic reactions and only slight to moderate local reactions followed the intracutaneous injections. Subcutaneous injections in doses of 100, 200, and 300 million bacilli, even 250, 500, and 1,000 million, produced some local and constitutional reactions, but much less than is commonly found with the standard subcutaneous doses of 500, 1,000, and 1,000 million.

Oral administration of three different preparations of mixed typhoid paratyphoid vaccines without preliminary bile treatment produced no significant rise in agglutinin titer when tested one month after ingestion.

These results suggest the greater use of the intracutaneous method to obtain immunization.

**TUBERCLE BACILLI, Analysis of Loewenstein's Investigations, Siegel, M. Am. J. M. Sc. 190: 435, 1935**

Of 911 blood specimens from 422 tuberculous persons at this hospital, there were 6 (14 per cent) macroscopic positive cultures and 55 (13 per cent) microscopic positive cultures by the Loewenstein technique. The macroscopic positive cultures were undoubtedly cultures of tubercle bacilli according to subculture and virulence tests. The microscopic positive cultures were not definitely proved to be cultures of tubercle bacilli, since subcultures and animal inoculation gave negative results.

The disparity between Loewenstein's results and those of other investigators still exists after five years of research.

Loewenstein's investigations may be criticized from two points of view: his definition of a positive blood culture and his conception of a control.

**BACTERIAL FLORA, Associated With Foreign Bodies in Trachea and Bronchi, Bucher, C. J. Am. J. M. Sc. 190: 409, 1935.**

Cultures seeded from pus bronchoscopically collected from the trachea and bronchi furnish more reliable data on the kind of microorganisms there, than those seeded from expectorated material.

The bacterial flora present in the air passages in which a foreign body is lodged does not materially change with the type of foreign body, its location or length of sojourn there, the age of the patient and degree of obstruction induced by it.

Other things being equal, one pathogen seems as capable as another of producing severe grades of infection in an obstructed tracheobronchial tree.

Quantitative differences in the numbers of colonies of different organisms on a plate of blood agar seeded from pus recovered from these lesions are a reliable guide in estimating the part that a given organism plays in the resulting infection.

The indications from a limited number of studies of the anaerobic bacteria from such lesions are that they are of minor importance.

The bacterial invasion and infection of the tissues of the lower respiratory tract at the site of a foreign body seem to follow the same general principles as those encountered in obstructive lesions elsewhere in the body. The irritation produced by the obstructing body itself and the amount of hindrance to drainage, primarily, and, secondarily, other factors favoring or hindering microbial multiplication such as the age and hardihood of the tissues, pressure on them and the duration of the irritation, determine and limit the infection.

**SCARLET FEVER, Convalescent Serum in, Joyne, A. L., Levinson, S. O., and Thalhimer, W. J. A. M. A. 105: 783, 1935.**

Of 862 home contacts who gave no history of scarlet fever and were passively immunized with convalescent scarlet fever serum, scarlet fever did not develop in 97.2 per cent.

Of 83 Dick-positive hospital contacts immunized with convalescent scarlet fever serum, scarlet fever did not develop in 95 per cent.

In immunized contacts in whom scarlet fever developed, it was usually in a modified form, believed to have been produced by partial immunization and resultant sero-attenuation.

Convalescent scarlet fever serum in adequate therapeutic doses administered early may abort the disease and usually causes recession of fever, diminution of toxemia and angina, and fading of the rash and appreciably shortens the period of illness.

Convalescent scarlet fever serum, directly or indirectly, either prevented the development of complications or reduced the frequency of their occurrence.

The influence of serum on late and complicated cases was less marked but frequently seemed beneficial.

By reducing the severity of the disease and the incidence of complications, the mortality rate was definitely diminished.

No unfavorable reactions, serum sickness, sensitization or anaphylactic shock were encountered with the use of human convalescent scarlet fever serum.

**PLAGUE, Rodent, in California, Kellogg, W. H. J. A. M. A. 105: 857, 1935.**

At present there is a lighting up of enzootic plague, which had become a commonplace, into a rather alarming epizootic, which is the most extensive outbreak of squirrel plague since the peak of the epizootic in Contra Costa and Alameda Counties in the period between 1907 and 1919. Other sharp outbreaks have doubtless occurred that burned themselves out with-

out the condition s being brought to attention in time to prove the nature of the epizootic. The present outbreak, however, has certain aspects that are not entirely reassuring. It is not without significance that after a considerable period of quiescence plague is found to be actively spreading among the wild rodent population of rural areas in widely separated districts and in areas far from any formerly known focus of infection. The prevalence of infected squirrels near the borders of Oregon and of Nevada and on the other side of the mountain range suggests that there is no natural limitation to the spread of plague through wild rodents to places far distant from its original entry into this country in the Bay district of California. Plague is very evidently a permanent problem on the Pacific Coast and the prospect of its becoming a problem in other states appears at the present time to be good. Especially to be feared so far as man is concerned is the pneumonic form of the disease, which, as has been indicated, may be directly related to plague in animals of the squirrel and ground hog type.

**MYELOMA Multiple, With Hyperproteinemia** Sweigert, C F. *Am J M Sc* 190 245, 1935

A case diagnosed clinically as multiple myeloma with hyperproteinemia is reported and the cases in the literature reviewed.

Hyperproteinemia is decidedly uncommon and occurs, in its most striking form, in multiple myeloma.

In the majority of instances it has been attributable to hyperglobulinemia without demonstrable relationship to Bence Jones protein. In three cases it was due to extraordinary amounts of Bence Jones protein in the serum. In the present case, both globulin and fibrinogen were increased in the blood plasma.

Hyperproteinemia may produce unusual and variable clinical phenomena, notably difficulty in counting erythrocytes, autohemagglutination, markedly accelerated sedimentation velocity of the erythrocytes, abnormal coagulability of the blood and spontaneous precipitation of protein in drawn blood. In the presence of Bence Jones proteinemia, precipitation in the serum may occur during inactivation for the Wassermann reaction.

The finding of hyperproteinemia or any of its manifestations should suggest multiple myeloma as a diagnostic possibility.

The clinical aspects of the blood and kidney changes in the present case are emphasized and their diagnostic importance is indicated. These include (a) a severe, progressive, macrocytic anemia with evidences of profound bone marrow disturbance and active regeneration, unassociated with achlorhydria and refractory to liver and iron therapy, and (b) an atypical nephropathy in which impairment of renal function without hypertension is a distinctive feature.

**CEREBROSPINAL FLUID During and Between Attacks of Migraine Headaches** Von Storch T J C, and Merritt H H. *Am J M Sc* 190 226, 1935

In 15 cases of migraine, the cerebrospinal fluid pressure, measured during a migraine headache, was slightly elevated in one case (190 mm of water), slightly low in 3 cases (40, 50, and 80 mm of water) and averaged 123 mm of water.

In 29 cases of migraine observed during an asymptomatic interval, the cerebrospinal fluid pressure was slightly elevated in 2 cases (185 and 190 mm of water), low normal in 3 cases (90, 90 and 95 mm of water) and averaged 139 mm of water.

In 43 determinations the total protein was normal in all fluids (exclusive of those contaminated by blood) irrespective of the presence of headache with the exception of 1 case which showed a normal value at a subsequent puncture.

In 10 instances, the amount of sugar present in the spinal fluid was within the normal range irrespective of the presence of headache.

In 7 fluids the chloride content was normal.

In all cases (44) the Ross Jones and Pandý tests and colloidal gold reaction were normal except when the spinal fluid was contaminated with blood subsequent to hemorrhage at the point of puncture

The cell count was normal (less than 6 cells per cmm) in all but 2 cases which had 6 and 11 cells per cmm, respectively (bloody fluids excluded).

In all of the fluids the Wassermann (or Kahn) test was negative

The authors observed no significant abnormality of, or consistent deviation from, the normal cerebrospinal fluid pressure in 44 cases of migraine. The total protein content, cytology and serology of the cerebrospinal fluid were normal. Any significant abnormality of the cerebrospinal fluid renders doubtful a diagnosis of migraine.

#### **LYMPHOPATHIA VENEREUM, Its Relation to Rectal Stricture, Vander Veer, J. B., Cormia, F. B., and Ullery, J. C. Am. J. M. Sc. 190: 128, 1935**

Of 47 cases of lymphopathia venereum infection here reported, 26 presented inguinal adenopathy and 21 rectal stricture. One case was complicated by esthiomene.

Lymphopathia venereum occurs with much greater frequency in the colored race.

Reading the Frei test after the fourth day is recommended.

The possibility of a diminution or absence of the Frei reaction in long standing cases is considered.

All patients with inguinal adenopathy of obscure etiology and those with rectal stricture should be tested with Frei antigen.

Cases presenting perirectal abscesses, fistulae in ano, or obscure pelvic infections should also be tested to eliminate the possibility of a lymphopathia venereum infection as an etiologic factor.

There is no specific treatment for the disease. Various methods have been considered.

#### **TRICHINOSIS, Diagnosis of, With Special Reference to Skin and Preceptin Tests, Spink, W. W., and Augustine, D. L. J. A. M. A. 104: 1801, 1935.**

Thirty five sporadic cases of trichinosis occurring in and around Boston during the past three years were analyzed.

The most reliable diagnostic clinical aid in these cases was the presence of an eosinophilia.

The skin test usually becomes positive about the seventeenth day of the infection, and the preceptin test usually at the end of the fourth week. These tests are of especial diagnostic aid in the early stages of the disease, when they are first negative and later become positive. Mild, sporadic and chronic cases of trichinosis were often detected only by these tests.

Other laboratory procedures, such as searching for the parasite in the stools, blood and spinal fluid, are time consuming, and the larvae are only rarely found.

#### **WASSERMANN-FASTNESS of Spinal Fluid in Treated Neurosyphilis, Goodman, M. J., and Moore, J. E. Arch. Int. Med. 55: 827, 1935.**

A study is presented of the incidence of clinical progression or relapse after prolonged treatment in 212 patients with neurosyphilis.

A comparison is made between two groups: 95 patients for whom the reaction of the spinal fluid remained persistently positive and 117 for whom the positive reaction was reversed by treatment.

Considering the groups as a whole, clinical progression occurred in 22 per cent of the Wassermann fast patients and in only 7 per cent of those with reversed reactions.

Limiting the study to patients treated for two or more years, subsequent progression occurred in 12.5 per cent of the Wassermann fast group and in 4.8 per cent of the group with reversed reactions.

Progression or relapse is more common in patients with parenchymatous neurosyphilis (tabes and dementia paralytica) than in those with nonparenchymatous types of neuroaxis involvement.

Even in a patient with apparently nonparenchymatous neurosyphilis, the subsequent relapse, if one occurs, is likely to be dementia paralytica in type

Of 31 patients in this series treated at some time with induced fever therapy (chiefly malaria), subsequent progression or relapse occurred in only 2

While there is a more definite relationship between the clinical outcome and the reaction of the spinal fluid in neurosyphilis than between the reversal or the fastness of the Wassermann reaction of the blood in various forms of late syphilis not involving the nervous system, a persistently positive reaction of the spinal fluid does not indicate the inevitability of subsequent progression or of relapse, nor can the rate or completeness of reversal of the reaction be used as the sole guide to the optimum duration of treatment in cases of neurosyphilis

**GLYCOSURIA, Factors Affecting the Appearance and Duration of, Robinson C S  
Derivaux, R C, and Hewell, B Am J M Sc 189 795, 1935**

A study of the relationships between hyperglycemia and glycosuria after the intravenous injection of glucose indicates that the excretion of sugar may be explained on the basis of the suggested participation of phosphorylation in the resorption of glucose from the glomerular filtrate

**TUBERCULOSIS Pathological Significance of the Leukocyte Reaction in Leukocytic  
Index With a Calculator to Facilitate Computation, Crawford, A M Am Rev  
Tuberc 31 611, 1935**

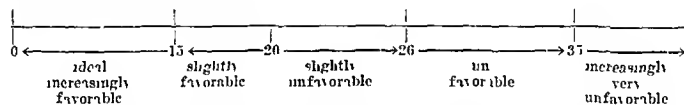
A calculator has been devised for quickly and accurately determining the  $\frac{N\%}{L\%}$  ratio value and for supplying the equivalent values for an abnormal elevation of monocytes and for abnormal total cell counts This calculator is shown below

From these values the leucocytic index is readily obtained for any given leucocytic picture

*The leucocytic index is obtained by adding*

- a The  $\frac{N\%}{L\%}$  ratio value
- b The value of abnormal monocyte percentages
- c The value of abnormal total cell counts

Interpretation of the index is given in terms of a favorable or unfavorable type of pathologic activity as follows



The leucocytic index is suggested as a short cut to correct interpretation of the leucocytic reaction in terms of favorable or unfavorable pathologic activity as evaluated by Medlar. It is only a conventional short hand symbol intended to represent the variation of the significant components of the leucocytic picture. The degree of pathologic activity having been determined by the index, it is urged that the components of leucocytic formulas be studied in order to get a complete understanding of the phase of the tuberculous process with which we are dealing. Medlar's conception of the significance of the role of each of the cell types in the pathogenesis of the disease may thus be readily and accurately applied and his conception of the pathologic status of the tuberculous process determined.



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## EDITORIALS

### Thoughts on the Education of the Public

WITHOUT doubt the greatest obstacle to advance in the fight against disease—to use a hackneyed figure and a frayed and stereotyped phrase—is public ignorance combined with credulity. Were it not for these the path of the quacks, the peddler of patent pills and lotions and, it must be said, the careless and haphazard physician, would be hard indeed.

So obvious has this been that for lo! these many years physicians as represented by national, state, and local organizations have striven valiantly to educate the public through the dissemination of information concerning disease, its recognition, its management, and its control.

The methods used have been varied and, with the advent of the radio, broadcasts along the lines of health-programs have come into being with increasing frequency.

Despite the time, effort, and money expended the results, however, would seem in some degree disappointing, and it is of some interest to speculate

upon the probable or, at all events, theoretical reasons why this should be so.

Is it because the public does not wish to be educated? Hardly, for popular texts on health and allied subjects seem to be enjoying a marked approval.

Is it because the methods used are faulty or their application is inappropriate or requiring a different handling or a different touch? The problem presents many difficulties, chiefly, perhaps, because it is extremely difficult to ascertain with any degree of approximate exactitude the proportion of the population who read popular articles on health or listen to "health broadcasts" and the degree to which they are influenced by either or both.

It seems fair to wonder if, perhaps, the earnest seeker after such information in the columns of his favorite newspaper may not experience some slight confusion of ideas if he happens to note or be aware of the fact that the same fount of medical knowledge which pours forth information in a happy and popular vein, may also, if the day and hour be appropriately chosen, be heard spouting in even a happier vein (for the words of the radio are golden words, indeed) on the merits of some patent remedy at so much per spout.

But perhaps the broadcast comes with the accolade of some medical organization of standing and repute—what then? Simply that, perhaps from some private and commercial station or in the pages of varied publications come such ominous descriptions of the "medical trust" that even then the program must penetrate a murky mist of distrust and disbelief before it can begin to educate or inform.

It is not at all unlikely that the first reaction excited in the mind of the listener might be to the effect: "Who is this fellow, anyway? Never heard of him!" I wonder what my own doctor would say about that?

The answer to that possibility would logically seem to be the County Medical Society broadcast where the voice is familiar, the reputation known.

If we are to be candid, however, it must be admitted that many such programs fail lamentably of the purpose for reasons not always clear or obvious. Sometimes because the subjects chosen are trite and stereotyped, sometimes because the manner in which they are presented smacks of the textbook and is devoid of popular appeal, and sometimes because the speakers are chosen, not for their aptitude for the purpose, but with the idea of giving as many members of the Society as possible their little moment of pseudo importance and acclaim.

It is the belief of the present deponent, who is, indeed, rather firmly convinced of the validity of the contention, that the disproportion between the efforts expended and the results evident in this matter of the education of the public are very largely based upon the truth expressed in the old adage which concerns the horse led to the trough.

Without doubt there is a public thirst for information in matters medical. Equally without doubt, there is a time and place when that desire is acute, when such information is not listened to simply as an unavoidable interim—happily brief—between orchestras, but when the entire attention of

the listener is focused on the problem, when, indeed, he goes out of his way in search of such information.

It may conceivably be somewhat difficult to interest an athlete in the mysteries of what is popularly known as "rheumatism." But this same subject is a matter of intense interest to the patient with joint-pains, just as food and its properties are of great interest to the woman eager to keep her girlish figure (overlooking the fact that she has become an older, if not wiser, matron), and of no interest (though it should be) to the individual who gets along nicely on three hearty meals a day, not to mention a nibble or two between and a little snack before going to bed.

Every patient wants to know, among other things, what is the matter with him, how he got that way, and why it makes him feel the way he does.

He may never put such questions articulately but the desire to know is there. And, if he is fortunate enough to encounter in his physician one who is able and willing to tell him—he will listen and try his darndest to absorb the information he receives. And if, in this way, he learns only one thing at a time, at least he *learns* that and thus may become a center for its further dissemination.

And his physician knows, too, that someone is actually listening to what he has to say, not languidly, not inattentively, not perforce, but eagerly and avidly.

And it is the further belief of this deponent that the information given in the doctor's office to those who are anxious to acquire it will in the long run do more for the education of the public in matters medical than the set programs all the audience of which are certainly not equally interested in hearing them.

And so, having stated his belief that the best and most appropriate place for the education of the public is the doctor's office, and in so proclaiming having suggested that the physician ask himself if he is fulfilling to its greatest degree what may well be regarded as an important function reacting to the benefit of physician and patient alike; and, if not—why not?—further this deponent sayeth not.

—R. A. K.

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### Automatic Euthanasia

IT HAS been many years since William Osler suggested humorously that it might be well to terminate life at a time when it is no longer useful and becomes a burden to the aging individual. This was meat for the press of the time, and was therefore widely publicized. Cartoons lampooned and ladies' clubs debated. Theologians objected, moralists argued, and biologists produced statistics.

The furor died down, the impracticability of such a procedure being generally agreed upon, and "euthanasia" persisted as a household word, its meaning well understood.

But have we done with euthanasia? The arbitrarily controlled arrangement implied in the name is, of course, not to be considered. But is NATURE herself slowly and quietly arranging things in accordance with the suggestion made by Su William?

Overpopulation scarcely seems a pressing problem at the present. There is so much of the world still uninhabited, and at the same time habitable. But overpopulation is not a world problem. It is usually one of a community, a country, or a geographic area or unit. War, famine, disease, and pestilence have prevented saturation in the past.

What is the present situation? We are faced with an economic problem in which those who have passed a certain age find it difficult to procure for themselves an adequate remuneration for services rendered. This is in essence a young man's world. The vast majority of aged individuals have failed to provide for their later years. They become burdens, if not to themselves, then to their children or the community. Even the efficient person past middle life finds it difficult to hold his position, much more so, to procure one.

The writer not long ago saw a laborer in his middle forties who surprisingly, dyed his hair. Upon questioning he explained that he was prematurely gray, and that as long as his hair was gray he had found it practically impossible to procure a job.

For the individual the ideal arrangement on this earth would be a proper provision for comfortable old age, free from worry, for everybody. Possibly the old age pensions recently inaugurated will help to this end, but at best they can provide little more than an escape from pauperism or dependency. Individualistically we cannot but hope for a comfortable old age with reasonable freedom from care. Biologically, however, there appear to be definite trends which promise to defeat the accomplishment of this objective.

The decline in infant mortality has increased the average duration of life. It has increased the population many fold. According to estimates, if there were no wars or epidemics, the present rate of increase would result in complete saturation of the world within one hundred years. At that time every tillable acre would be needed to provide sustenance, assuming there occur no new discoveries in chemistry to provide synthetic foods or otherwise simplify the problems of nutrition.

Assuming that such an eventuality is not desirable, what steps are likely to eneuvent it? Both control and the elimination of the unfit, preferably through sterilization and eugenic measures, appear ideal. Since, however, they interfere either with personal privilege or the ambitions of nations they will be slow indeed in gaining general acceptance and popularity.

War and disease, especially epidemic disease, remain as the most effective measures of combating the population increase which has resulted from the saving by science of infant and child life. We might add the automobile.

There may be another factor, of tantamount importance. We speak with regret of the good old days in which life was no such bustle hurry, and confusion as it now is. We recall when competition was not so keen—when there was still time for the amenities—when life, though less speedy, was

enjoyed more in the living of it. Today's life of competition is one of stress and strain, drive and fatigue, and, all too often an early demise. Competitive effort and modern ways of living make it so. There is no escape, if one would achieve what is known as success.

Longevity of the individual has not been increased. Indeed there is evidence of an increased incidence of the degenerative diseases of middle life and the advancing years. If the strain of the modern day continues, there appears every probability that among those who reach maturity, the average life expectancy will be distinctly shorter than it has been. Men will continue to burn themselves out—to "kill themselves with overwork." And nothing that science can do will prevent it as long as civilization continues at its pace. An entire revamping of the manner of living of the whole world would be necessary. But with continued population increase, with every increasing competition, this is almost an impossibility.

Our great-grandfathers, pioneers and the like, died chiefly from the action of extrinsic factors—factors external to the body itself. Such were bullets, animals, bacteria, poisons, extreme heat or cold, starvation, injury and the like. Today, while these lethal factors continue active, we must add important contributory causes, intrinsic in origin. Other things being equal, the low speed engine will outlast the high speed machine.

All of which is little more than saying that modern life is a man-killing process.

Can we take exception to this? Is it really objectionable? To the individual, yes, very much so. Each and every one of us is imbued with the desire to live on and on. Biologically, there might be room for argument.

At what stage is man's function on earth completed? Warthin recognized three phases: (a) preparation for independent existence, which covers the period from birth through adolescence; (b) a period of twenty or more years during which the chief biologic function is the reproduction of the species; and (c) a period in which man protects his progeny until the latter is ready for independent existence. After the third phase the cycle is completed and man's function is terminated. There is no longer need for continued existence of the individual. One might argue that mental activity continues to grow or improve even after general physical involution has begun. This is probably true, and represents the only important reason for continued existence of the individual (a minority of individuals) after the biologic need for his living has ceased.

But this is of little interest to NATURE.

We are faced, therefore, with a situation in which we must recognize that NATURE may be quietly but inexorably exercising that function which Osler once suggested as possibly desirable. As individuals we cannot like it, but in the abstract we must admit that it is a logical counteractant to man's folly in persisting, with little reason, in his efforts to overpopulate the earth.

—W. T. V.

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### THE REACTION OF NORMOBLASTIC BONE MARROW TO LIVER EXTRACT\*

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OLIVER P. JONES, PH D., MINNEAPOLIS, MINN

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RECENTLY there appeared an article by Jacobson<sup>1</sup> describing the reticulocyte response of normal guinea pigs to the oral and parenteral administration of liver extracts. His experiments were performed in an attempt to find a suitable method for assaying the potencies of the various therapeutic substances used in the treatment of macrocytic hyperchromic anemias. The work is similar to that of Vanghan, Muller, and Zetzel<sup>2</sup> and Vaughan, Muller, and Minot<sup>3</sup> with pigeons. It is maintained that the pigeon and the guinea pig respond to various liver extracts because they normally possess a megaloblastic bone marrow comparable to that found in the human being with pernicious anemia during relapse, and that it is abnormal for the pigeon to possess a normoblastic bone marrow.<sup>1, 4, 5</sup> It is claimed further that the failure of a reticulocyte response in rats to substances effective in treating pernicious anemia is due to the absence of a megaloblastic bone marrow.<sup>7</sup> In a recent monograph Singer<sup>8</sup> has shown conclusively that not only does the normal rat respond to injections of liver extract but also to injections of unconcentrated human gastric juice. Since rats will not elicit a reticulocyte response to gastric juice from pernicious anemia patients, Singer maintains that this method can be used as a biologic test for pernicious anemia. Recently Wills and others<sup>10</sup> have discredited the above conclusion regarding the reticulocyte

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enjoyed more in the living of it. Today's life of competition is one of stress and strain, drive and fatigue, and, all too often an early demise. Competitive effort and modern ways of living make it so. There is no escape, if one would achieve what is known as success.

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At what stage is man's function on earth completed? Warthin recognized three phases: (a) preparation for independent existence, which covers the period from birth through adolescence; (b) a period of twenty or more years during which the chief biologic function is the reproduction of the species; and (c) a period in which man protects his progeny until the latter is ready for independent existence. After the third phase the cycle is completed and man's function is terminated. There is no longer need for continued existence of the individual. One might argue that mental activity continues to grow or improve even after general physical involution has begun. This is probably true, and represents the only important reason for continued existence of the individual (a minority of individuals) after the biologic need for his living has ceased.

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—W. T. V.

suitable in any respect, he can propose a new, better term, but he should not create confusion by the false use of the original name "

Some of the confusion concerning the megaloblast normoblast problem is due to the various techniques used<sup>17-19</sup> From our own experience it is felt that the methods used by Doan and Zeifas<sup>20</sup> and Isaacs,<sup>21</sup> which depend on the emulsification of the marrow, are adequate for use in an attempt to make a differential count of marrow cells However, we do not believe that they bring out the finer cytologic detail of the nucleus as well as the dry "abklatsch" or imprint preparations stained with May Grunwald Giemsa The use of sections only (Turnbull<sup>22</sup>) has the disadvantage of not demonstrating the delicate nuclear structures in the very young cells, as pointed out by Downey<sup>24</sup> The inadequacy of supravital staining as used by Doan, Cunningham and Sabin<sup>18</sup> and Doan<sup>20</sup> in studying the finer morphologic details is discussed by Hall<sup>25</sup> We find that the imprint or "abklatsch" method used by Pappenheim, Ferrata, Naegeli, and Downey is the best suited for studying the cellular relationships of bone marrow components

Our experiments show there is a significant reticulocyte response by the guinea pig to parenteral liver extract as described by Jacobson<sup>1</sup> but that this response cannot be attributed to a megaloblastic bone marrow of the guinea pig, since we have shown that this marrow is normoblastic rather than megaloblastic In human beings those possessing a pathologic bone marrow, of the type found in pernicious anemia, respond to liver extract, while, on the other hand, in guinea pigs the normoblastic (proerythroblastic) bone marrow responds The response to the same antipernicious anemia principle in different species of animals not having a megaloblastic marrow emphasizes the fact that generalizations concerning the reticulocyte response to liver extract in pernicious anemia cannot be carried over to the other mammals without obtaining substantial morphologic evidence for the similarity of the corresponding hematopoietic tissue Since it cannot be shown that the test animals possess a megaloblastic bone marrow identical with that of pernicious anemia patients, we must conclude that the response is not due to a similarity of the bone marrows, but rather to an inexplicable species difference morphologically unexplained at present

We find no morphologic evidence for a megaloblastic bone marrow in the guinea pig The earliest forms of the red cell series in this animal are identical with those found in the normal human adult In spite of the fact that the reticulocyte response in the normal guinea pig to oral or parenteral administration of liver extract is conditioned by a totally different type of bone marrow than in human beings with pernicious anemia, this method of assay of the therapeutic potencies of various substances may still prove to be of great practical value

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#### ADDENDA

Just recently Jacobson has published an article which reiterates his speculations concerning the cause for the reticuloocyte response in guinea pigs to substances effective in treating pernicious anemia (*J. Clin. Investigation* 14: 664, 1935). He maintains that "The large number of megaloblasts, in proportion to the number of normoblasts, simulates the classical picture of the bone marrow findings in pernicious anemia." In other words, the guinea pig normally possesses a bone marrow which is encountered only under pathologic conditions in the human being.

In order to illustrate the differences between these two bone marrows and to show that they are not identical nor simulate one another, reference should be made to Figs. 1 and 2. The center cell in Fig. 1 is a promegaloblast, while the

two adjacent cells are basophilic megaloblasts. These cells are in no way related to the definitive red blood cell series nor are they found in normal marrow, whether it be guinea pig or human being.<sup>15</sup> The field in Fig. 2 represents two cells belonging to the first developmental stage of the red blood cell series found in normal guinea pig bone marrow. These cells are pronormoblasts (proerythro-

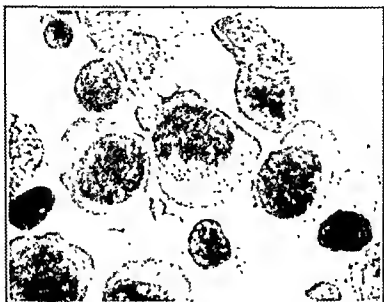


Fig. 1.—The center cell is a promegaloblast, while the two adjacent cells are basophilic megaloblasts. This is dry imprint preparation of biopsied pernicious anemia bone marrow during relapse. May-Grünwald-Giemsa stain. Magnification  $\times 1500$ .

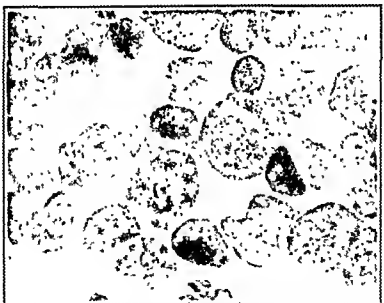


Fig. 2.—The cells in the center of the field are pronormoblasts (proerythroblasts) from a dry imprint preparation of normal guinea pig bone marrow. Nucleoli are present in both cells. Stain and magnification are the same as in Fig. 1.

blasts) and have been misinterpreted as "megaloblasts" by many authors. Note the difference in arrangement of chromatin as well as the nucleoplasmic ratio (in the megaloblasts and normoblasts). The cells found in Fig. 2 are identical to the first developmental stage of the red blood cell series encountered in normal human bone marrow.

# STUDIES OF PLASMA PROTEINS AND CHOLESTEROL\*

## IN NORMAL WHITE AND COLORED INDIVIDUALS, AND IN NEGROES WITH ARTERIOSCLEROSIS

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### INTRODUCTION

WHEN the medical experience of one of us began to include more clinical examinations of negroes some two or more years ago, it was apparent that arteriosclerosis was encountered both more frequently and at a much earlier age than in white patients. A study, recently completed, was made of the incidence of this finding in patients under the age of forty who presented no evidence of hypertension nor nephritis. Naturally, the question of etiology of vascular changes in young negroes arose. Since inadequacy of nutrition seemed possible in the face of the circumscribed diet of so many negroes, we began a study of the cholesterol and proteins of the blood plasma.

Fortunately, two of us had already made a study of these plasma components in a group of thirty-five healthy medical students. This group offered a satisfactory control for the studies on thirty-one negroes, one-half of whom presented arteriosclerosis, while the other half were apparently well-nourished young negroes complaining of minor disturbances.

### EXPERIMENTAL

All determinations were made on oxalated plasma, using the minimum amount of dry potassium oxalate necessary to prevent clotting. All analyses were started within a few hours after the blood samples were taken; in a few instances, duplicate determinations were carried out. If there seemed to be any reason for the determination being inaccurate, it was either discarded or repeated.

The plasma proteins were determined after the method of Wu<sup>1</sup> as modified by Andersch and Gibson.<sup>2</sup> This modification seemed to be very satisfactory in our hands, and where duplicate determinations were made, the agreement was all that could be desired. The plasma cholesterol was determined by the method of Myers and Wardell<sup>3</sup> as modified by Andes.<sup>4</sup> In most cases whole blood cholesterol was also estimated as a check. In Table I are given the results of plasma proteins and cholesterol in thirty-five healthy white persons. All but two were either medical students or technicians, between the ages of eighteen and twenty-nine years. Twenty-six were males. Two white females, aged forty-one and

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fifty years, were included in this group. Practically all of this group had been examined physically by members of the Department of Medicine at the beginning of the school year. Urine examination and blood pressure determinations in all were negative. The results of this study are summarized at the end of Table I.

The results of plasma protein and cholesterol determination in sixteen negro males showing arteriosclerosis are presented in Table II. All of the patients were ambulant and attended the Medical Out Patient Department of Charity Hospital. Vascular disease was judged on the degree of sclerosis, incompressibility and tortuosity of the radial and brachial arteries and in some, of the dorsalis pedis artery. Visible pulsation of the radial arteries appeared rather frequently. The ages of the group ranged as follows: one was sixteen years of age, eleven fell between the ages of twenty to thirty years, and four were between the thirtieth and fortieth years.

TABLE I  
BLOOD CHOLESTEROL AND PLASMA PROTEINS OF NORMAL INDIVIDUALS

NO	AGE AND SEX	PLASMA CHOLESTEROL	PLASMA PROTEINS				A/G RATIO*
			TOTAL	FIBRINOGEN	ALBUMIN	GLOBULIN (SERUM)	
1	20 M	159	7.63	0.27	4.83	2.53	1.73
2	18 F	193	8.19	0.33	5.37	2.50	1.90
3	20 M	103	7.13	0.30	4.00	2.53	1.27
4	26 M	188	8.50	0.52	5.01	3.06	1.37
5	20 M	179	8.42	0.27	5.56	2.59	1.94
6	25 M	170	7.69	0.31	4.49	2.69	1.50
7	22 M	172	8.51	0.32	5.73	2.66	2.00
8	18 M	140	8.30	0.29	5.06	2.04	1.02
9	23 M	185	7.25	0.30	4.45	2.50	1.50
10	22 M	162	8.59	0.31	5.52	2.76	1.60
11	25 M	142	8.93	0.38	5.27	3.28	1.44
12	24 M	220 <sup>†</sup>	8.15	0.23	4.06	2.06	1.55
13	20 M	141	8.04	0.21	4.88	2.95	1.34
14	26 M	174	8.77	0.37	5.60	2.80	1.77
15	19 F	188	7.73	0.32	5.06	2.35	1.90
16	24 F	107	7.67 <sup>‡</sup>		5.04	2.63	1.72 <sup>‡</sup>
17	24 M	175	7.67 <sup>‡</sup>		5.16	2.63	1.76 <sup>‡</sup>
18	22 M	151	7.25 <sup>‡</sup>		4.60	2.65	1.56 <sup>‡</sup>
19	29 M	173	7.87 <sup>‡</sup>		4.06	2.91	1.55 <sup>‡</sup>
20	27 M	140	7.69	0.25	4.53	2.91	1.43
21	26 F	175	6.97	0.36	3.85	2.56	1.32
22	18 M	145	8.01	0.35	4.78	2.88	1.48
23	22 M	160	7.40	0.24	4.68	2.48	1.72
24	20 F	160	8.77	0.28	5.16	3.33	1.43
25	23 M	148	8.47	0.27	5.37	2.83	1.73
26	24 M	158	7.27	0.27	4.37	2.63	1.51
27	23 M	144	8.79	0.33	4.96	3.50	1.30
28	25 F	151	9.15	0.31	5.16	3.68	1.30
29	24 M	193	7.79	0.25	4.78	2.76	1.59
30	26 M	146	8.17	0.34	5.30	2.53	1.85
31	22 M	199	7.82	0.37	4.69	2.76	1.50
32	25 F	150	8.83	0.37	5.27	3.76	1.34
33	41 F	203	7.62	0.48	4.45	2.69	1.40
34	23 F	150	8.63 <sup>‡</sup>		5.17	3.46	1.38 <sup>‡</sup>
35	50 F	207	8.27	0.20	5.40	2.67	1.88
High	50	207 (220)	9.15	0.52	5.00	3.68	2.00
Low	16	140	6.97	0.20	3.85	2.00	1.27
A <sup>‡</sup>	24	167	8.06	0.32	4.94	2.95	1.56

\*Where G represents serum globulin and fibrinogen (plasma globulin).

<sup>†</sup>Value is for total serum proteins.

<sup>‡</sup>Calculated assuming the fibrinogen to be 0.30 gm.

<sup>‡</sup>This figure is excluded in the final average.

observed in the average values in both groups). The fibrinogen and serum globulin values are definitely increased, while the albumin is lowered. This produces a fairly marked reduction in the A/G (albumin/globulin) ratio. In fact, the ratios are similar to those found in mild nephritic and nutritional edema (see Table IV), though no history nor evidence of edema was present. Furthermore no marked difference is present between the group of normal negroes and those with arteriosclerosis. The total proteins and fibrinogen values are almost identical, while the A/G ratio is slightly lower in the arteriosclerosis group.

Explanation of the findings in the negroes is at this time impossible. Patients were selected who were apparently well nourished. It is evident to everyone that the diet of the average Southern negro is not as well balanced as the accepted dietary demand. At first an attempt was made to gain information about the diet from each patient. This was soon abandoned, however, because of the low mental state and lack of cooperation of so many of the patients. Therefore, an idea of the average diet of the negro was obtained by questioning the more cooperative patients, from the information given by a rural storekeeper, and from Dr. P. B. Cameron, who conducts the Diabetic Clinic at Charity Hospital. These sources all agree on the constituents of the negro diet. Fresh meat is available only once or twice a week, at the most, and then only in small quantities. Eggs are used to some extent; also milk, though the latter, in the urban districts, may be of poor quality. Carbohydrate is high in the diet, in the form of grits, cornbread, white bread, rice, and red and navy beans. The leafy vegetables are represented mainly by collard, mustard or turnip greens. Calorie intake and the distribution of the above constituents of the diet no doubt vary in the individual cases on the basis of economic considerations.

#### COMMENT

The results of our studies are of especial interest since they may indicate a nutritional lack before it is manifest clinically, a "nutritional pre-edema state." We feel that these findings should be called to the attention of students interested in serum protein studies. Our results show that nutritional deficiency may make itself manifest by a change in the serum proteins, although not of such degree as to lower the serum osmotic pressure sufficiently to produce edema.

In order to compare our findings with those found in nutritional edema, we have arranged our average figures together with those of several other investigators (Table IV).

It is apparent that the total protein concentration in our negro groups is higher than in the normal, as a result of the increase in the globulin fraction. Since the decrease in the albumin is not very marked, the globulin increase has maintained the osmotic pressure high enough so that edema cannot occur. In fact, if the fibrinogen is added to the calculations of osmotic pressure, the pressures of the normal white and the negro groups are almost identical (40.7 and 39.1 cm. of water, respectively). It would seem, therefore, that in our patients the fall in albumin has started as a result of certain dietary deficiencies, but that a compensatory globulin increase has occurred which keeps the osmotic pressure approximately normal.

This is in contrast with most of the other values reported in malnutrition (as shown in Table IV), although all cases with edema show some lowering in the A/G ratio, as a result of a slower fall in the globulin fraction. It would seem that our cases fall in the region between the normal and those persons with frank protein shortage.

In explaining nutritional edema, others have discussed the matter of total nitrogen intake. We should like to view the question from a slightly different angle.

TABLE IV  
AVERAGE SERUM PROTEIN VALUES

TYPE OF CASE	AUTHORS	NO OF CASES	SERUM PROTEINS			A/G RATIO*	OSMOTIC PRESSURE IN CM H <sub>2</sub> O†
			TOTAL	ALBU MIN	GLOBU LIN		
Normal white individuals	Andes, Kampmeier, and Adams	35	7.74	4.94	2.85	1.73	39.1
Normal negroes	Andes, Kampmeier, and Adams	15	7.85	4.34	3.52	1.23	36.9
Negroes with arterio sclerosis	Andes, Kampmeier, and Adams	16	7.79	4.25	3.54	1.20	36.2
Nutritional edema	Youmans and associates <sup>9</sup>	31	6.95	3.98	2.89	1.38	31.2
Malnutrition without edema	Bruckman and Peters <sup>10</sup>	41	6.17	3.82	2.43	1.57	27.1
Malnutrition with edema	Bruckman and Peters <sup>10</sup>	18	5.75	3.16	2.64	1.20	23.0
Malnutrition without edema	Bruckman, D'Esopo and Peters <sup>11</sup>	42†	6.20	3.86	2.41	1.60	27.4
Malnutrition with edema	Bruckman, D'Esopo and Peters <sup>11</sup>	18†	5.50	3.16	2.69	1.17	22.0

\*Where G = serum globulin

†Calculated according to the formula of Wells, Youmans and Miller<sup>12</sup>

‡A larger number of cases were analyzed for the total proteins

Since bread and beans make up such a large proportion of the diet of the negro, the total quantity of protein consumed is probably adequate in all cases. However, the biologic value of these proteins is not very high [52 per cent for white bread and 38 per cent for beans<sup>13</sup>]. Also a consideration of the amino acid content of the proteins of white bread and brown beans reveals the fact that white bread proteins are deficient in cystine and lysine, and bean proteins are deficient in cystine. In view of the fact that serum globulin contains only about one half as much cystine and two thirds as much lysine as does albumin,<sup>14</sup> it seems plausible to believe that the body can more easily synthesize globulin than albumin, when the food source is deficient in these two amino acids. This would explain why the plasma globulin is increased, even though the albumin has decreased.

Since the osmotic pressure of globulin is much lower than albumin, any osmotic deficiency due to lack of albumin is made up only by an increase in globulin several times the magnitude of the albumin deficit. This would account for the high total proteins.

In addition, the diet is unquestionably low in vitamins A, C, and D, and the absence of these accessory food factors may be a factor in the genesis of the abnormal protein concentration.

The small differences between the plasma protein figures in normal negroes and those with arteriosclerosis are not great enough to be very significant, although a difference exists. Furthermore, any relation between plasma proteins and arteriosclerosis cannot be regarded as established, although the figures presented do not in any sense rule out such a possibility.

#### SUMMARY

1. Values for the plasma protein constituents and plasma cholesterol are given for 35 normal white persons, 16 young negroes with arteriosclerosis and 16 young negroes without evidence of vascular disease.

2. Both the arteriosclerosis and normal negro groups showed findings for the above constituents so similar as to be considered as a single group, the arteriosclerosis group showing the greater deviation from the normal.

3. The principal changes in the negro patients were (a) slightly increased total proteins; (b) definitely increased fibrinogen and serum globulin; and (c) lowered albumin concentration.

4. The A/G ratio was therefore markedly decreased, being similar to, though not as marked as, that commonly observed in nephritic or nutritional edema. However, nephritis and nutritional edema were not present in our material.

5. The results of this study suggest a "nutritional pre-edema" stage.

6. The abnormal protein findings are explained on the basis of a qualitative protein deficiency, although the explanation is merely a hypothesis on our part.

7. Cholesterol values were essentially the same in all three groups, no significant alteration being found in the group with arteriosclerosis.

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## THE RELATION OF THE THYROID GLAND TO HEMATOPOIESIS\*

### I EXPERIMENTAL TOTAL THYROIDECTOMY IN THE RABBIT

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WE BECAME interested in the relationship of the thyroid gland to hemato-  
poiesis for the following reasons (1) The close clinical similarity between chronic lymphatic leucemia and the exophthalmic type of hyperthyroidism (2) The experimental work of Gottlieb<sup>1</sup> who showed that the number of lymphocytes in the blood of the thyroid vein was 30 per cent higher than that in the blood of the thyroid artery or the systemic capillary system (3) The report of a case of lymphatic leucemia in which a total ablation of the thyroid gland was performed with startling disappearance of the signs of the disease (4) The development of anemia in spontaneous and postoperative myxedema

*Thyroid Gland and Leucemia*—Clinically, chronic lymphatic leucemia and hyperthyroidism are very much alike Symptoms which are characteristic of both diseases include fatigue, loss of weight, tachycardia, perspiration, and tolerance to cold, they are, of course, more prominent in hyperthyroidism As in exophthalmic goiter, leucemic patients may also present such symptoms as an increased appetite, flushing of the skin, diarrhea, menstrual disturbances, nervousness, irritability, and insomnia Bilateral exophthalmos has even been reported by Friedgood<sup>2</sup> in five of his ten cases of lymphatic leucemia Minot and Means<sup>3</sup> had previously called attention to the presence of unilateral exophthalmos in leucemia, and in addition, to a fine tremor of the hands which they attribute to the associated anemia Generalized lymphadenopathy and splenomegaly, so characteristic of lymphatic leucemia, are present, but to a lesser degree, in 30 per cent of the cases of hyperthyroidism<sup>4,7</sup> The etiology of the enlargement of the lymph nodes and spleen is not known, but it is probably a part of the generalized lymphoid hyperplasia which also gives rise to the characteristic and well-known pathologic picture of lymphoid hyperplasia seen in the thyroid gland of exophthalmic goiter Further evidence of this lymphoid activity is reflected in the study of the blood The Koehler<sup>8</sup> blood picture, viz, leucopenia and relative lymphocytosis, is still accepted by most clinicians<sup>1,6,7,9,11</sup> Menkin<sup>12</sup> again recently stressed the finding of relative lymphocytosis in 67 per cent of the cases of hyperthyroidism that he studied There is a difference of opinion as to what effect iodine has on the blood picture of hyperthyroidism Jackson<sup>13</sup> states that it has no effect, Hertz and Leiman<sup>9</sup> that it has a definite depressing influence on the mononuclear elements It is significant, however, to note that all agree that thyroidectomy restores the normal differential formula of the blood<sup>9,11,17</sup> Caro<sup>13</sup>

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described a case of fatal exophthalmic goiter with such atypical findings in the blood that he thought he was dealing with an associated pseudoleucemia. He later collected several other cases showing similar blood pictures. It is not necessary to discuss the characteristic blood picture of lymphatic leucemia, but we think it pertinent to point out a rough similarity in the relative lymphocytosis of hyperthyroidism and of the aleucemic phase of lymphatic leucemia. The basal metabolic rate is increased not only in hyperthyroidism, but also in cases of both lymphatic and myelogenous leucemia.<sup>3, 14, 15</sup> In fact, Friedgood<sup>16</sup> has observed an increase in the basal metabolic rate in certain cases of acromegaly, diabetes mellitus, essential hypertension, polycythemia vera, and pernicious anemia. Just as the cause of the increased oxygen consumption in hyperthyroidism is, for the most part, speculative, so is the explanation of the increased metabolic rate in leucemia. It has been shown that the height of the basal metabolic rate in leucemia is not necessarily related to the height of the leucocyte count, but more closely comparable to the patient's general condition, the pulse rate, the degree of anemia and inversely to the number of platelets of the blood.<sup>14, 15</sup> Some authorities believe that in leucemia the level of the basal metabolic rate is of more prognostic value than the number of the leucocytes.<sup>15</sup> The well-defined and favorable effect of Lugol's solution in exophthalmic goiter is also seen, but to a lesser degree, in leucemia. Friedgood<sup>17</sup> has noted a definite decrease in the nervousness and in the fatigue of leucemic patients following the administration of Lugol's solution. Such favorable effects of iodine as reductions of the basal metabolic rate, the pulse rate, the lymphocytes of the blood and the size of the enlarged lymph nodes and spleen are variable. In a certain percentage of patients, there was a definite symptomatic remission, but in others there was no effect. The action of Lugol's does not seem to be due to either the reduction of the basal metabolic rate, or any influence exerted by the thyroid gland, but through a "sedative" effect on a hyperactive state of the sympathetic nervous system. So, in summing up a comparison of the two diseases, chronic lymphatic leucemia and exophthalmic goiter, we find not only many similar signs and symptoms, but, in addition, that both have generalized lymphoid hyperplasia, that both have an increase in circulatory lymphocytes, and that both have an increased basal metabolic rate which responds beneficially to Lugol's solution. The main difference in these factors in the two diseases, as pointed out by Friedgood,<sup>17</sup> is not qualitative but rather quantitative.

*Thyroidectomy and Leucemia.*—The suggestion of total thyroidectomy for the treatment of lymphatic leucemia may appear, at first glance, to be the result of a wave of impulsive, enthusiastic surgery that usually follows any new procedure such as has recently been advanced by Blumgart and his associates<sup>18</sup> in the treatment of angina pectoris and congestive heart failure. However, from the foregoing discussion there is evidence to indicate that removal of the thyroid gland in lymphatic leucemia might have a beneficial effect, certainly enough to warrant its therapeutic trial in this otherwise fatal disease. Dameshek and coworkers<sup>19</sup> reported such a case in a forty-two-year-old woman with all the signs of an aleucemic lymphatic leucemia with a basal metabolic rate of plus 65. It was thought that the continued loss of weight, profuse drenching sweats, increasing nervous

symptoms, and the beginning circulatory failure might be due to the markedly increased metabolic rate. When the patient became extremely ill and failed to respond to rest in bed, Lugol's solution and x-ray therapy, complete ablation of the thyroid gland was performed. Following this procedure the metabolic rate dropped strikingly, the clinical signs and symptoms of hypermetabolism disappeared as did the signs of circulatory failure. The patient gained weight, and the lymph nodes and spleen regressed about 90 per cent from their original size. There was complete disappearance of all the patient's symptoms, and the blood picture became almost normal. Dameshek<sup>20</sup> reports that she is "continuing to be healthy" seventeen months after the operation. This amazing result commands attention and invites further clinical trial cautiously carried out.

*Thyroid Gland and Lymphocytosis*—Gottlieb<sup>1</sup> in studying the differential counts of blood taken from the thyroid vein of six cases of hyperthyroidism, noted the lymphocytic ratio was increased 33 per cent when compared with that taken from the blood of the thyroid artery and capillary bed. This observation seemed to indicate that a large number of lymphocytes were added to the blood as it circulated through the thyroid gland. Considering the findings of the pathologists that in hyperthyroidism there is a general lymphatic hyperplasia with lymphatic foci in the thyroid gland itself, Gottlieb assumed that this increase of the lymphocytes in the thyroid vein must have been due to an actual formation of lymphocytes in the thyroid gland. However, Menkin<sup>21</sup> felt that the reason for the relative lymphocytosis in hyperthyroidism was due to a sympathetic stimulation of the lymphoid tissues, particularly to the contraction of the spleen, which caused a discharge of lymphocytes into the blood. It has been shown by Hurvey<sup>21</sup> and Menkin<sup>22</sup> that contraction of the spleen by the use of drugs or emotional excitement, may increase the lymphocytes of the blood. In our minds, the reason for the lymphocytosis in hyperthyroidism has not been answered, and we are now studying this problem but have not, as yet, formed any definite conclusions.

*Thyroid Gland and Anemia*—Anemia associated with myxedema is a well known clinical observation that has received much comment in the literature.<sup>23-27</sup> The classification of the anemia and its treatment has been the cause of considerable variance of opinion. Lerman and Means<sup>23</sup> reported that 50 per cent of their patients with myxedema have a gastric anaecidity, and that anemia is much more common in that group than in those showing free acid. Although the anemia improved in a few instances as the myxedema was treated, they urged the addition of iron in adequate doses to bring about a more rapid improvement in the blood. Stone<sup>24</sup> felt the anemia was the result of a depression of erythropoiesis which in turn is a part of a generally diminished function shared by the other tissues. MacKenzie<sup>25</sup> concurs with the belief that the anemia is simply a manifestation of the effect on the bone marrow of the sluggish oxidation that occurs in all tissues.

#### EXPERIMENTAL INVESTIGATION

*Problem*—With these clinical facts and observations in mind, and with comparatively few references to investigative work in this field, we made the following studies. We felt that a complete removal of the thyroid gland in ani-

mals would give us information as to (1) the occurrence and type of anemia that develops in myxedema, and (2) the effect of total ablation of the gland on the normal differential count.

*Experimental Procedure.*—Four albino rabbits six months old were subjected to a complete thyroidectomy, under ether anesthesia. No signs of infection or tetany developed during the postoperative course. A control series consisted of four rabbits of the same age and weight, two of which were subjected to ether anesthesia and a simple incision of the neck exposing but leaving the thyroid gland intact. This was done in order to insure against any immediate effect on the blood that might arise from either the general anesthesia or surgical incision. Control and thyroidectomized animals were kept together in the same

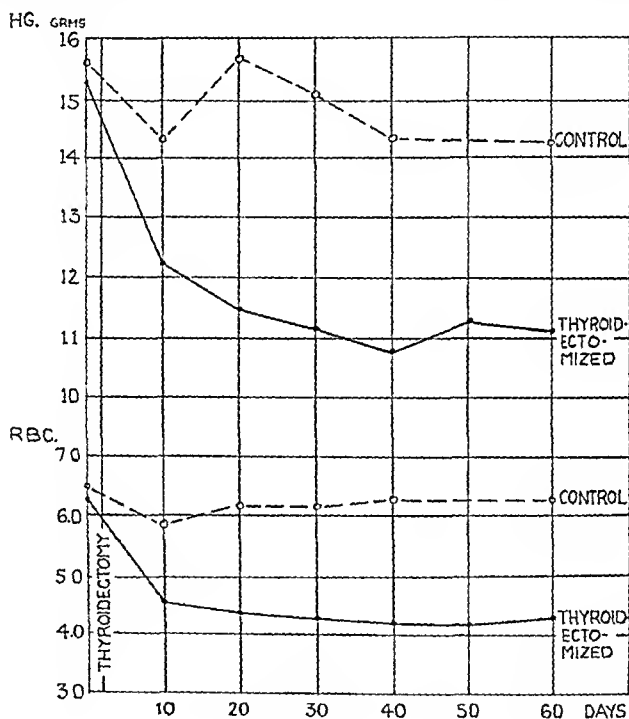


Fig. 1.—Hemoglobin and red cell counts of normal and thyroidectomized animals.

pens and on the same standard diet. Weekly records were kept of their weight. Blood counts were done before and at ten-day intervals following the operation. The blood was taken from the ear, during fasting periods of constant length, and at the same time of day, in order to minimize any error that might result from the normal rhythm of the leucocytes.<sup>28</sup> The rabbits were accustomed to being handled, and made little or no resistance.<sup>22</sup> Hemoglobin determinations were done by means of a Sahli hemoglobinometer (17 gm. equals 100 per cent), and standardized pipettes were used in the determination of the number of the red and white blood cells. Fixed smears were taken on cover slips, stained by Wright's method, and a hundred cells counted on each of a pair of cover slips. Hematoerit determinations were done with the Wintrobe tubes, using the method as devised by him.<sup>29, 30</sup>

*Experimental Observations*—During the first ten day period following operation, there was a definite, sharp drop in both the hemoglobin and red blood cells (Fig 1). A further, but slower, decrease continued for the first month, after which time the hemoglobin and red blood count remained at about the same level. The control series showed only a slight decrease in the hemoglobin and red cell count, and after the first ten day interval, both returned and remained within normal limits.

These observations are consistent with the findings of Essex,<sup>31</sup> Kishi,<sup>32</sup> and Mansfield.<sup>33</sup> However, Kunde and his associates,<sup>34</sup> working with much younger rabbits (cietins), did not notice any significant change in the number of red blood cells until four or five weeks following thyroidectomy. After that time, an anemia developed of moderate and persistent degree. Their findings agree with

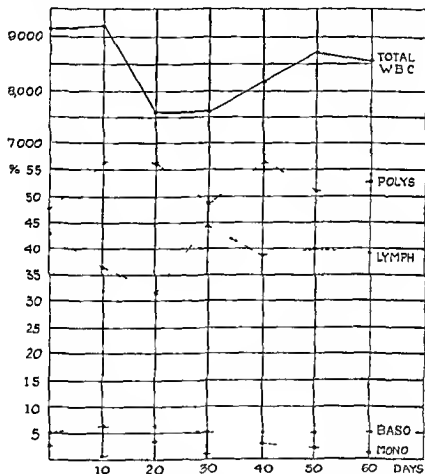


Fig 2—White blood cell and differential counts of thyroidectomized animals

onits, however, in stating that the anemia is characterized by a color index of plus one and over, and a red cell that is larger than normal. The results of our hematocrit readings are tabulated in Table I. Our normal readings check closely with Wintrobe's observations on rabbit's blood.<sup>35</sup> It is interesting to note that the size of the normal red cell of the rabbit is only about three fourths that of the human red cell. In the thyroidectomized group, there is a definite increase in the mean corpuscular volume; there is a very slight increase in the mean corpuscular hemoglobin and no change in the mean corpuscular hemoglobin concentration. These findings are characteristic of a microcytic anemia.

The number of white cells showed a delayed slight reduction that reached its lowest level during the second to third week, but remained within normal limits (Fig 2). A study of the differential formula during each ten day interval

TABLE I  
HEMATOCRIT READINGS

	NORMAL		THYROIDECTOMIZED
	WINTROBE	AUTODORS	
Hemoglobin, grams	13.02	14.7	11.2
Red blood cells, per c.mm.	6.29	6.15	4.38
Volume packed red cells, c.c.	39.79	38.80	30.50
Mean corpuscular volume, c. microns	63.70	61.70	70.50
Mean corpuscular hemoglobin, micromicrograms	21.00	23.50	26.50
Mean corpuscular hemoglobin concentration, per cent	33.20	38.10	37.00
Sedimentation rate, mm./hr.		1.0	1.0

revealed, except for slight minor fluctuations, no significant change from the normal.

The sedimentation rate of the red cells of the rabbit is very much slower than in the human being. The normal rate of a control series was 1 mm. per hour. There was no change in the sedimentation rate of the thyroidectomized animals.

There was a steady gain in weight of the myxedematous animals, and with the exception of some loss of hair, and a definite decrease of activity, the animals appeared to be otherwise healthy.

In addition to the hematologic observations, complete studies of the blood chemistry were followed, and these, with studies of the effect of thyroid feeding on the anemia, and with final pathologic studies of the spleen, lymph nodes, and bone marrow, will be reported at a later date.

#### SUMMARY

1. Hematologic observations are reported on four normal rabbits and four rabbits in which a complete thyroidectomy had been performed.
2. A moderate and persistent anemia rapidly developed after the removal of the thyroid gland. The anemia is of the macrocytic type.
3. There is a very slight, transient decrease of the white blood cell count; no significant change in the differential formula.
4. There is no change in the sedimentation rate following thyroidectomy.

We wish to thank Miss Mildred Braden for her very valuable technical assistance.

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## ATTEMPTS TO APPLY THE ACETYLENE METHOD OF DETERMINING THE CARDIAC OUTPUT TO THE DOG\*

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IT HAS not been a great many years since estimations of human blood pressure first came into use as an important criterion of the circulatory state. More recently it has become possible to determine accurately the amount of blood expelled by the heart. The various methods now available for the determination of the cardiac output undoubtedly will advance the understanding of circulatory problems quite as much as did the use of the sphygmomanometer. These methods for the determination of cardiac output are no longer simply laboratory procedures; they are used in the clinic by both internist and surgeon.

In man the Grollman acetylene method<sup>1, 2</sup> gives remarkably constant values for the output of the heart under any constant condition. In experimental animals the Fick method is commonly used to determine the cardiac output. This method, however, not infrequently gives wide fluctuations in the results when numerous repeated determinations are desired. These fluctuations seem to be inherent in the method. Perhaps the puncture of the heart with its disturbance to the rhythm of the heart beat produces some of the deviations, but the withdrawal of a mixed sample of arterial and venous blood is the common source of error. This paper records the attempts to apply the acetylene method of determining the cardiac output to the dog.

Certain conditions must be fulfilled in order to obtain reliable results with the acetylene method. It must be possible to produce a homogeneous mixture between the air-acetylene mixture in the gas container and in the alveoli. There must be perfect equilibrium between the acetylene tension in the alveolar air and in the blood passing through the lungs at the time of taking samples of the alveolar air. Two adequately spaced alveolar samples must be secured before the completion of a single circulation of the blood.

Difficulty was encountered in having the dog breathe so that satisfactory mixtures of the gases could be obtained. Several unsuccessful attempts were made to force the dog to breathe deeply and quickly from a spirometer by stimulation of the saphenous or sciatic nerves. A mechanical method of respiration was then devised by which the gas mixture was forced back and forth between a bag and the alveoli through a tube in the dog's trachea.

The container for the gas mixture is illustrated in Fig. 1. The top (a) and base (b) of the container were cylindrical pieces of wood 13 cm. in diameter, around which was fitted heavy rubber to form the body of the bag (c). From opposite sides of the base were two openings to the outside, (d) to admit the gas mixture and (e) to connect with a three-way aluminum valve (f). A coil spring (g) on the inside kept the bag in the expanded position

\*From the Laboratory of Surgical Research, Western Reserve University.  
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unless forced down by the handle (*h*) Copper capillary tubing (*i*) lead to sampling tubes for collection of alveolar air All connections were hermetically cemented The bag held approximately one liter of air

The three way valve (*f*, Fig 1) was in turn, attached by air tight connections to a tube in the dog's trachea In the sacrifice experiments a large, heavy walled rubber tube was used and a ligature passed around the trachea In the survival experiments a metal tube (*a*, Fig 2) 12 mm in diameter was found to be satisfactory A copper capillary tube (*b*) inside the metal tube (*a*) provided a means of infusing by a pressure bulb (*c*) a piece of a finger

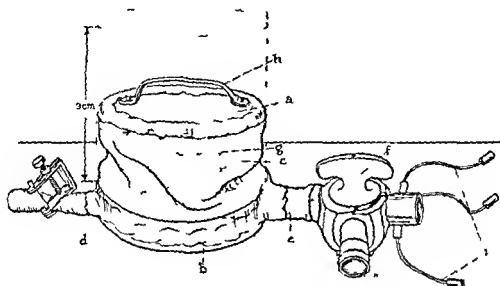


Fig 1

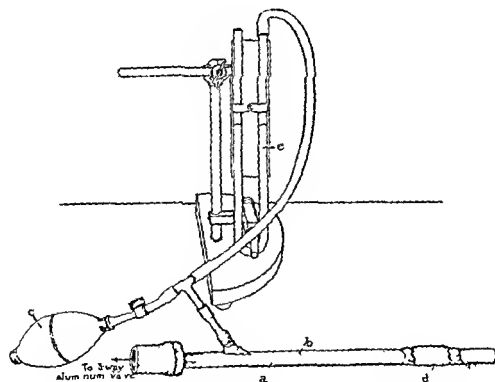


Fig 2

from a surgical rubber glove (*d*) which was tied and hermetically sealed around the large tube, so that no air could escape around the tracheal tube an absolutely necessary condition A mercury manometer (*c*) was inserted so that any leakage might be instantly detected

In order to determine the time necessary to establish equilibrium between the acetylene in the alveolar air and in the blood, simultaneous blood and alveolar samples were taken at various intervals after the beginning of rebreathing the air acetylene mixture The carotid artery was cannulated and the blood samples



were collected over mercury in an air-tight chamber coated with potassium oxalate. Alveolar samples were collected in evacuated sampling tubes. Alveolar samples were analyzed for acetylene tension on the Haldane gas analysis apparatus (11 c.c. burette) as described by Grollman<sup>2</sup> and blood samples were analyzed for acetylene tension on the Van Slyke apparatus by the method described by Grollman, Proger and Dunning.<sup>3</sup> Since there is some error inherent in the method of analysis, particularly of the gas sample, equilibrium was considered to have been established when the tensions in the simultaneous blood and alveolar samples agreed within 10 per cent.

When the samples were taken exactly fifteen seconds after the beginning of rebreathing the gas mixture, in 7 out of 9 experiments the acetylene tension in the blood and lungs agreed within 10 per cent; there was agreement within 10 per cent in 7 of 9 experiments when the samples were taken after more than 15 seconds, namely, at 16, 17, 18, 19, 20, 23 and 28 seconds; but in samples obtained under 15 seconds, namely 10, 12, 13 and 14 seconds, there was agreement within 10 per cent in only 3 out of 9 experiments.

Our data indicate that by fifteen seconds from the beginning of rebreathing the acetylene tensions in the alveoli and blood are usually in equilibrium. There may be equilibrium in some instances by fourteen or even thirteen seconds from the beginning of rebreathing; but, since it is not possible to predict when this will be true, it is not safe to collect the first sample before the fifteenth second after the beginning of rebreathing.

Attempts were made to obtain equilibrium more quickly by increasing the rate of the rebreathing. Although we do not have sufficient data on this point to justify definite conclusions, our results lead to the belief that the optimal rate is one complete respiration approximately every two seconds.

As noted above, it is essential that the final sample be procured before the completion of a single circulation of the blood. Starr and Collins<sup>4</sup> have found that in the dog an average of 35 per cent of the blood put out by the left heart has returned to the right heart within fifteen seconds. It would seem improbable, therefore, that a correct value for the output would be obtained from calculations based upon the difference in gas content of two samples, one of which was procured fifteen seconds and the other more than fifteen seconds (twenty-two to twenty-eight seconds) after the beginning of rebreathing.

In five different dogs, the results of cardiac output tests obtained by the acetylene method were compared with the results obtained by the direct Fick method. In all of our experiments the dogs were in a basal condition. After determination of the oxygen consumption, the heart was immediately punctured for blood samples for the Fick test, and usually within fifteen minutes (twenty-five minutes in one test) the indirect test was carried out.

Fig. 3 shows that in every instance the output computed by the acetylene method is much too low. The output, as determined by the acetylene method was 32 per cent less in one dog, 48 per cent less in another, and more than 50 per cent less in the other three than the results obtained by the direct Fick method indicate.

On three dogs, under morphine plus pentobarbital anesthesia, series of tests by the acetylene method were carried out on different days. There was a wide variation in the output values obtained for each dog, although the animal was in as nearly identical condition (basal) as possible for all tests. For example, in one case there was a 28 per cent difference between the largest and the smallest values obtained for the minute output, in another instance there was a 35 per cent difference and in another a 30 per cent difference between the two extreme values.

The cardiac indices obtained on these animals (the cardiac output per minute per square meter of body surface) also show great variation, and they are

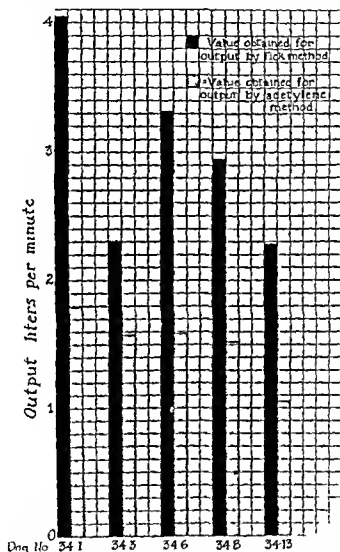


Fig. 3—Comparison of values for the cardiac output of the dog by the Fick and by the acetylene methods

much too low. One of us (Williams), using the Fick method, has calculated the cardiac index on a series of seventeen normal dogs and found it to average 3.95. The average value obtained on our dogs by the acetylene method is only 2.15 (average 46 per cent too low).

#### SUMMARY

Simultaneous blood and alveolar samples taken from the dog at various intervals after the beginning of forced rebreathing of an air-acetylene mixture show that a requisite equilibrium between the acetylene tension in the alveolar air and in the blood can be attained. As a rule, such equilibrium was not estab-

lished before fifteen seconds of rebreathing at the approximate rate of a complete respiration every two seconds. This fact is not favorable to the acetylene method, since a second requisite for its validity is the procuring of at least two adequately spaced samples before the completion of one circulation, and since investigations have been reported (Starr and Collins<sup>4</sup>) indicating return of blood to the heart in such a short period (fifteen seconds) that obviously it would be impossible to obtain two samples within such a period if the first sample is not secured before the fifteenth second.

A comparison of the values obtained for the cardiac output of five dogs by the acetylene method with the values obtained by the direct Fick method shows that the results obtained by the acetylene method are nearly 32 per cent too low in one case and about 50 per cent too low in each of the other four cases.

Tests on the same dog by the acetylene method give wide variation in results, which would preclude its use for comparison of output values before and after production of a circulatory disturbance.

Comparison of the average cardiac index calculated from the acetylene tests (2.15) with the average found in a series of normal dogs by the Fick method (3.95) indicates that the index by the acetylene method is much too low (average 46 per cent).

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## THE PREVENTION OF THE ANAPHYLACTIC SHOCK DUE TO HORSE SERUM BY THE INJECTION OF BCG\*

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IT HAS previously been shown by one of us (E M F<sup>1</sup>) that bronchial asthma was associated with pulmonary tuberculosis in about 15 per cent of the series studied. Similar reports have been made by other workers in this field.<sup>2-4</sup> That the incidence of tuberculosis of the lungs is lower among asthmatic individuals than among other groups of subjects has been frequently emphasized,<sup>5-7</sup> but we cannot agree with the view that has been put forward by other workers that there is a fundamental antagonism between the two conditions. The nonspecific desensitization with small doses of Old Tuberculin for the treatment of asthma and other allergic conditions as proposed by Storm van Leeuwen is suggestive in this connection.

It was shown by Seligmann<sup>8</sup> (1912), W. Pagel and Garcia Frias<sup>9</sup> (1930), Friedberger and Gajzago<sup>10</sup> (1930), and W. Pagel<sup>11</sup> (1931) that tuberculosis infection in guinea pigs provides protection against anaphylactic shock, and it was demonstrated by W. Pagel and Garcia Frias that this is not due to a specific tuberculous allergy. This protection is not dependent on the spread of the foci, as subcutaneous injection is sometimes more effective than intravenous injection although the latter provides a greater number of foci. The shock resistance was also transmissible from one infected animal to one noninfected.

Friedberger and Gajzago found that tuberculous infection in guinea pigs did not afford protection in all cases, but that the result depended upon the shocking dose of protein. Small doses, effective in control animals, were ineffective, but larger doses of protein still produced shock. Pagel explains the protection produced by tuberculous infection as being due to a failure of antibody response to the sensitizing dose since the injection of a serum containing antibody is followed by a diminution of the protective power.

*Experimental*—In the experiments reported we have investigated the effect of the injection of Calmette's BCG into sensitized guinea pigs. This seemed of interest and importance from both the clinical and theoretical points of view.

Different series of guinea pigs were sensitized with horse serum and a few days later treated with BCG before the intravenous injection of serum. The controls were sensitized and later on desensitized with the same dose of the same horse serum, but of course without the intermediate injections of BCG. Both the time and dose have been varied as may be seen in the tables. Sensitization was carried out with 0.05 cc of horse serum and increasing

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doses of B.C.G. emulsion in saline were injected from the eighth day. After a series of doses (subcutaneous and intraperitoneal) during ten days, the animals were left from two to four weeks, and then desensitized by intravenous doses of fresh horse serum.

TABLE I\*

ANIMAL NO.	DATE AND SENSITIZING DOSE	DATE AND B.C.G. INJECTIONS	DATE AND DESENSITIZING DOSE	EFFECT
1	May 2, 1933 0.02 c.c. serum	May 9 and 19, 1933 5 s.c. 1.1 P.	June 13, 1933 0.5 c.c. serum	No shock
2	May 2, 1933 0.02 c.c. serum	May 9 and 19, 1933 5 s.c. 1.1 P.	June 13, 1933 0.5 c.c. serum	No real shock
3	May 2, 1933 0.02 c.c. serum	May 9 and 19, 1933 5 s.c. 1.1 P.	June 20, 1933 0.8 c.c. serum	No shock
4	May 2, 1933 0.02 c.c. serum	May 9 and 19, 1933 5 s.c. 1.1 P.	June 20, 1933 0.8 c.c. serum	No shock
Control 1	May 2, 1933 0.02 c.c. serum	—	June 2, 1933 0.4 c.c. serum	Shock and death
Control 2	May 2, 1933 0.02 c.c. serum	—	June 5, 1933 0.5 c.c. serum	Shock and death
Control 3	May 2, 1933 0.02 c.c. serum	—	June 13, 1933 0.5 c.c. serum	Shock followed by recovery

\*As controls for experimental animals 3 and 4 see Table VII, Nos. 4 and 5; 0.8 c.c. of serum followed by shock and death.

TABLE II

ANIMAL NO.	DATE AND SENSITIZING DOSE	DATE AND B.C.G. INJECTIONS	DATE AND DESENSITIZING DOSE	EFFECT
1	Sept. 1, 1933 0.02 c.c. serum	Sept. 9 and Sept. 22, 1933 5 s.c. 1.1 P.	Oct. 10, 1933 0.6 c.c. serum	No shock
2	Sept. 1, 1933 0.02 c.c. serum	Sept. 9 and Sept. 22, 1933 5 s.c. 1.1 P.	Oct. 10, 1933 0.6 c.c. serum	Shock and death
3	Sept. 1, 1933 0.02 c.c. serum	Sept. 9 and Sept. 22, 1933 5 s.c. 1.1 P.	Oct. 11, 1933 0.8 c.c. serum	Shock and death
4	Sept. 1, 1933 0.02 c.c. serum	Sept. 9 and Sept. 22, 1933 5 s.c. 1.1 P.	Oct. 11, 1933 0.8 c.c. serum	No shock
5	Sept. 1, 1933 0.02 c.c. serum	Sept. 9 and Sept. 22, 1933 5 s.c. 1.1 P.	Oct. 13, 1933 0.5 c.c. serum	Some convulsions. No typical shock
6	Sept. 1, 1933 0.02 c.c. serum	Sept. 9 and Sept. 22, 1933 5 s.c. 1.1 P.	Oct. 13, 1933 0.5 c.c. serum	No shock
Control 1	Sept. 1, 1933 0.02 c.c. serum	—	Oct. 10, 1933 0.6 c.c. serum	Shock and death
Control 2	Sept. 1, 1933 0.02 c.c. serum	—	Oct. 11, 1933 0.8 c.c. serum	Shock and death
Control 3	Sept. 1, 1933 0.02 c.c. serum	—	Oct. 13, 1933 0.5 c.c. serum	Shock and death

*Table I*—Three of the animals infected with BCG developed no shock and one, although definitely disturbed gave no real signs of anaphylactic shock. The controls, injected on the same days as the infected animals with the same doses of horse serum, gave the typical shock leading to death with the exception of Control 3.

*Table II* shows a similar experiment in which, however, the shock doses were first used on the controls so that no doubt could be entertained that a lethal dose was being employed. The two BCG animals with shock had the same picture at autopsy as the controls—emphysema of the lungs, dilated heart, and no coagulation of the blood. It thus seems that repeated BCG

TABLE III

ALL ANIMALS IN THIS TABLE SENSITIZED WITH 0.1 CC OF HORSE SERUM ON JUNE 6, 1933.

ANIMAL NO	DATE AND BCG INJECTION	DATE AND DESENSITIZING DOSE	EFFECT
1	July 8 and July 31 1933 0.05 0.1 mg BCG 4 I P 2 cc	July 31, 1933 0.3 cc serum intravenously	No shock. Died later from intraperitoneal abscess.
2	July 8 and July 31 1933 0.05 0.1 mg BCG 4 I P 2 cc	Aug 4, 1933 0.5 cc serum intravenously	No shock.
3	July 8 and July 31 1933 0.05 0.1 mg BCG 4 I P 2 cc	Aug 14 1933 0.2 cc serum intra arterial	No shock.
4	July 8 and July 31 1933 0.05 0.1 mg BCG 4 I P 2 cc	Aug 14 1933 0.2 cc serum intra arterial	Prolonged shock.
5	July 8 and July 31 1933 0.05 0.1 mg BCG 4 I P 2 cc	Aug 14 1933 0.2 cc serum intra arterial	Prolonged shock.

TABLE IV

ALL ANIMALS IN THIS TABLE SENSITIZED WITH 0.2 CC OF HORSE SERUM ON JULY 8, 1933.

ANIMAL NO	DATE AND BCG INJECTION	DATE AND DESENSITIZING DOSE	EFFECT
1	July 4 to 14 1933 4 I P 2 cc	July 30 1933 0.3 cc serum intravenously	No shock.
2	July 4 to 14 1933 4 I P 2 cc	Aug 4, 1933 0.5 cc serum intravenously	No shock.
3	July 4 to 14 1933 4 I P 2 cc	Aug 4 1933 0.5 cc serum intravenously	No shock.
4	July 4 to 14 1933 4 I P 2 cc	Aug 14 1933 0.2 cc serum intra arterial	Heavy shock.
	July 4 to 14 1933 4 I P 2 cc	Aug 14 1933 0.2 cc serum intra arterial	Heavy shock.

injections into guinea pigs produce protection against anaphylactic shock in the limits above shown, namely when the desensitizing dose is not far above the lethal quantity.

The following experiments, which were carried out with the assistance of Dr. W. Pagel, were designed to examine the effect of variations of time, sensitizing dose, and injection route (intra-arterial).

*Tables III and IV.*—We investigated the effect of the time of sensitization (before and after B.C.G. treatment) and of the doses for the sensitizing injection.

TABLE V

CONTROLS FOR TABLES III AND IV  
ALL ANIMALS IN THIS TABLE SENSITIZED WITH 0.2 C.C. OF HORSE SERUM ON  
JUNE 24, 1933

ANIMAL NO.	B.C.G.	DATE AND DESENSITIZING DOSE	EFFECT
1	—	July 31, 1933 0.3 c.c. serum intravenously	Shock and death
2	—	Aug. 4, 1933 0.5 c.c. serum intravenously	Shock followed by recovery
3	—	Aug. 14, 1933 0.5 c.c. serum intravenously	Shock and death
4	—	Aug. 14, 1933 0.5 c.c. serum intravenously	Shock and death

The sensitizing dose taken was 0.2 c.c. of horse serum. The animals were injected with B.C.G. four times subcutaneously and twice intraperitoneally. The animals were desensitized with an intra-arterial injection of horse serum. Three of the animals died after immediate shock and one recovered from a very heavy shock.

The five animals sensitized prior to the injection of B.C.G. (Table III) did not develop a fatal shock, one dying later of an intraperitoneal abscess. Three of these animals were desensitized by means of intra-arterial injection; two of these had a prolonged but not deadly shock.

The other series (Table IV) with B.C.G. injections prior to the sensitization showed in three cases no shock and in two cases a heavy but not deadly shock. It would seem that it is immaterial whether the injections of B.C.G. are commenced before or after sensitization, but that the protection afforded is less efficient when a more effective method of desensitization is used (intra-arterial).

In another experiment we determined whether a single small dose of B.C.G. would afford the same protection against the same shock. Some animals were sensitized prior to the injection of B.C.G. (Table VII) and others subsequent to the B.C.G. (Table VI).

*Tables VI and VII.*—In both series one guinea pig developed no shock, and in Table VI one guinea pig gave a typical shock but without death. In all other

cases a typical anaphylactic shock with death ensued. It may thus be seen that a single small injection of BCG affords no protection against anaphylactic shock. It is to be observed that in the two injected animals in which shock occurred there was no difference from ordinary shock in the gross symptoms or in the postmortem findings. We must thus consider that as in Friedberger's experiment with tuberculous infection the protection afforded by BCG infection is not complete and may fail in a considerable proportion of cases.

TABLE VI

ALL ANIMALS IN THIS TABLE WERE SENSITIZED WITH 0.2 CC OF HORSE SERUM ON MAY 9, 1933

ANIMAL NO	DATE AND BCG INJECTION	DATE AND DESENSITIZING DOSE	EFFECT
1	May 2, 1933 0.1 mg sc	May 31, 1933 0.5 cc serum intravenously	No shock
2	May 2, 1933 0.1 mg sc	June 16, 1933 0.4 cc serum intravenously	Shock and death
3	May 2, 1933 0.1 mg sc	June 16, 1933 0.4 cc serum intravenously	Shock followed by recovery

TABLE VII

ALL ANIMALS IN THIS TABLE SENSITIZED WITH 0.2 CC OF HORSE SERUM ON MAY 2, 1933

ANIMAL NO	DATE AND BCG INJECTION	DATE AND DESENSITIZING DOSE	EFFECT
1	May 9, 1933 0.1 mg sc	May 31, 1933 0.4 cc serum intravenously	Shock and death
2	May 9, 1933 0.1 mg sc	June 2, 1933 0.25 cc serum intravenously	No shock
3	May 9, 1933 0.1 mg sc	June 5, 1933 0.4 cc serum intravenously	Shock and death
4	May 9, 1933 0.1 mg sc	June 20, 1933 0.8 cc serum intravenously	Shock and death
5	May 9, 1933 0.1 mg sc	June 20, 1933 0.8 cc serum intravenously	Shock and death

In the infected animals which died in shock, postmortem examination showed enlarged lymph glands in the neck and thorax but no other sign of BCG infection.

Some unrecorded experiments have been carried out with BCG cultures which were attenuated and which produced no local lesions after injection, as well as some experiments with an emulsion of bacilli which had been heated



to 56° C. for thirty minutes. These experiments showed that in this case there was no protection whatsoever against the anaphylactic shock.

Experiments where a local abscess was produced with staphylococci gave no immunity to shock. Some other experiments have been tried with sensitization toward extracts of pollen and goose feathers, but no real sensitization could be obtained with these materials.

#### CONCLUSIONS

1. Repeated doses of B.C.G. emulsion in saline produce a state in the sensitized animal which protects it from the anaphylactic shock due to horse serum.

2. With the desensitizing or shocking dose much in excess of the fatal dose, the protection becomes less effective.

3. Single small doses before or after sensitization do not induce the state of protection against serum shock.

4. The injection of attenuated or killed bacilli or the production of abscesses with other bacteria does not induce any degree of protection.

We wish to acknowledge the collaboration of Mr. J. W. Walker in this work.

The investigation was commenced in the department of the late Professor Calmette in the Institut Pasteur, Paris. We are indebted to Professor Calmette for facilities for work and for subsequently supplying us with cultures of B.C.G. at the Westminster Hospital where the work was continued with the aid of the John Profit Bequest for Tuberculosis.

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## A STUDY OF THE EFFECTS OF VACCINE INJECTIONS UPON SKIN SENSITIVITY\*

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CERTAIN changes in the degree of skin sensitivity to intradermal vaccine injections have been seen to occur as a result of vaccine therapy. This paper records some observations of such changes and our ideas regarding their significance. Vaccine skin reactions have been such useful guides to us in diagnosis and treatment that we believe that we are justified in presenting the results of this study. These results appear to us to add support to our opinion that autogenous vaccine skin reactions are significant and specific phenomena.<sup>1</sup>

The types of vaccine skin reactions as already described by us,<sup>1, 2</sup> are (1) a wheal appearing a few minutes after the intracutaneous injection of a vaccine and (2) a tuberculin type of skin reaction which reaches its maximum development in about forty eight hours.

1 The early or wheal areola type of skin reaction differs in no wise from that following the intradermal injection of pollen or other protein extracts in sensitized patients.

2 The late local skin reaction, on the other hand, is produced, read, and interpreted so differently by various observers that confusion exists in regard to its exact nature. In order to assist in interpreting this reaction, we repeat here our conception of it. We have defined it as being a lesion of the skin beginning about six hours after and at the site of the intracutaneous injection of not less than 0.01 cc. nor more than 0.02 cc. of a 1 per cent volumetric suspension of killed bacteria in normal saline solution and 0.25 per cent trikresol and persisting for forty eight hours or more. Such a lesion is usually composed of a palpable nodule overlaid by a deep red macule and surrounded by a pink areola. Tenderness and local heat may be present as well as other features that are named in Table I.

The data upon which this paper is based were obtained from the case histories of thirty three patients suffering with allergic symptoms associated with infection, in other words cases of bacterial allergy (Table II). In addition to other examinations of these patients a bacteriologic survey was made of all of their accessible foci of infection, of all abnormal discharges and, in some cases of the feces. A vaccine of each recovered organism was prepared according to an established technique and was accurately standardized.<sup>3</sup> The patients were then tested intracutaneously with each autogenous vaccine and afterward received therapeutic injections of those organisms which had produced positive reactions. The duration of vaccine treatment

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varied from about three months to three years. Inoculations were made intracutaneously in testing and either intraeutaneously or subintracutaneously in treatment.\* In each of these two methods the resulting local reactions are easily observed throughout the course of treatment. The intensity and duration of the late local reactions were used as guides in the selection, not only of the vaccines to be used, but also of the amount of dosage. At some time during or at the end of treatment, new tests were performed in each patient with some of the original vaccines.

TABLE I  
FEATURES OF THE LATE LOCAL VACCINE REACTION

NO.	FEATURE	AS RECORDED
1	Nodule	N
2	Redness (macule)	R
3	Areola	A
4	Swelling	S
5	Tenderness	T
6	Pain	Pa
7	Heat	H
8	Ecchymosis (icterus)	E
9	Pruritus	Pr
10	Pustule	P
11	Desquamation	D
12	Pigmentation	Pi
13	Lymphangitis	L
14	Lymphadenitis	G

TABLE II  
CLINICAL DIAGNOSES OF THIRTY-THREE PATIENTS

Allergic coryza	5
Allergic coryza and aphthous stomatitis	1
Asthma	12
Asthma and bronchiectasis	2
Asthma and chronic bronchitis	3
Asthma and emphysema	1
Asthma and pollinosis	1
Asthma and urticaria	1
Arthritis (chronic rheumatoid and mixed)	6
Arthritis, rheumatoid, and mucous colitis	1
	<hr/> 33

The changes observed in the skin sensitivity of these patients to some of their autogenous vaccines are recorded in Tables III and IV. In each case, these changes were measured by comparing the results of the original tests with those of the retests. Such changes, when they occurred, are recorded in terms of the early and late local reactions.

One hundred and six tests before treatment and the same number of retests after treatment were made with the 106 vaccines listed in Table III. All of these vaccines were used as treatment material. It is seen that skin sensitivity as manifested by the early local reaction remained unchanged after treatment in 67 per cent, was decreased in 23 per cent, and increased in

\*A subintracutaneous infection is one which deposits the vaccine into the deeper layers of the skin and into the subcutaneous tissue immediately adjacent thereto. In making such an injection, the point of the needle, after puncturing the skin, is turned back into the under surface of the skin and at this point the syringe is emptied.

10 per cent. On the other hand, skin sensitivity as manifested by the late local reaction (bacterial allergy) remained unchanged after treatment in 17.9 per cent, was decreased in 72.6 per cent, and increased in 9.5 per cent.

Sixty-two tests and the same number of retests were made with the sixty-two vaccines listed in Table IV. None of these vaccines were used in treatment. As in the case of those vaccines which had been used in treatment, but few changes were noted in skin sensitivity of the early or wheal areola producing type. Quite a different state of things is seen, however, in regard to the type of skin sensitivity manifested by the late local reaction. Here, only about 40 per cent of the retests were followed by reactions indicating diminished skin sensitivity in contrast to a regression in skin sensitivity to 72.6 per cent of the vaccines used in treatment (Table III).

Thus it is seen that the early type of skin reactivity to the majority of the vaccines, whether they were used in treatment or not, persisted unchanged after treatment. This fact seemed to bear no relationship to the presence or absence, or to the degree of clinical improvement. The delayed or tuberculin type of skin sensitivity, however, appeared to have been changed in a definite manner following vaccine treatment. Skin tests after treatment with those vaccines which were used in treatment were followed in most cases by reactions which indicated that reduction in bacterial allergy had occurred.\* Thus reduction moreover, was proportionate to the degree of clinical improvement of the patient at the time of retest. In the case of those vaccines which were not used in treatment, retests were followed by late local reactions which, on the whole, showed no consistent changes in bacterial allergy. These facts are brought out in Tables V and VI which are summaries of Tables III and IV, respectively. Here the thirty-three patients are divided into groups according to the degree of clinical improvement present at the time of retest. The early type of skin reactivity was found to be unchanged after 60 per cent or more of the retests in all groups in both tables. Delayed skin sensitivity was diminished, in the case of the vaccines used in treatment, after 85 per cent of the retests in patients who were completely relieved or markedly improved, after 66 per cent of the retests in those who were moderately improved, and after 38 per cent of the retests in those who had little or no improvement. In contrast to these findings, skin sensitivity, after retests with the vaccines not used in treatment, was found to be either unchanged, decreased or increased in an irregular manner, not consistent with the presence or absence of clinical improvement.

Some of the skin reactions observed in two individuals are represented in graphic form in Charts 1 and 2. The early or wheal reactions to each organism are recorded by sketches approximately the size and shape of the reactions on the patient's skin (see upper part of Chart 1). In cases in which the wheal was surrounded by an areola the fact is indicated by the letter A within the sketch. The late local reactions are represented in a quantitative manner by block graphs, in which each block stands for a separate feature or component part of the reaction (see Chart 2 and the lower part of Chart 1). At the left side of each section of the groups of blocks is a letter identifying

\*Since this paper was written Wainwright has reported similar observations (Wainwright Chas. W. *The Treatment of Chronic Rheumatoid Arthritis with Streptococcus Vaccine on the Basis of Skin Sensitivity* J. A. M. A. 105: 1357, 1934).

TABLE III

CHANGES IN THE SKIN SENSITIVITY OF THIRTY-THREE PATIENTS TO THE VACCINES WITH WHICH THEY WERE TREATED, EXPRESSED IN TERMS OF THE EARLY AND LATE LOCAL REACTIONS

PATIENT	CONDITION AT TIME OF RETEST	VACCINES USED IN TESTS AND IN TREATMENT	EARLY LOCAL REACTION	LATE LOCAL REACTION
1 S. A.	Markedly improved	Atypical gram neg. coccus <i>Streptococcus viridans</i>	U U	D† D
2 A. H.	Moderately improved	<i>Staphylococcus aureus</i> <i>Staphylococcus aureus hem.</i>	U I‡	D D
3 B. M.	Markedly improved	<i>B. coli communior</i> <i>Micrococcus catarrhalis</i> <i>Streptococcus hemolyticus</i> <i>B. coli communis</i>	U U U U	D D D U
4 R. P.	Moderately improved	<i>Staphylococcus albus</i> <i>Streptococcus hemolyticus</i> <i>Streptococcus viridans</i>	I U U	U D U
5 C. B.	Completely relieved	<i>Micrococcus catarrhalis</i> <i>Staphylococcus albus</i> <i>Staphylococcus albus hem.</i> <i>Streptococcus viridans</i>	U D D U	U D D D
6 J. S.	Completely relieved	<i>B. alkaligines</i> <i>Staphylococcus aureus</i> <i>Streptococcus viridans</i> <i>Streptococcus viridans</i>	I U U D	D D D D
7 A. W.	Slightly improved	<i>Micrococcus albus</i> <i>Staphylococcus aureus</i> <i>Streptococcus hemolyticus</i> <i>Streptococcus viridans</i>	U U U D	D D D D
8 A. V.	Markedly improved	<i>Streptococcus viridans</i> <i>Streptococcus hemolyticus</i> <i>Micrococcus catarrhalis</i> <i>B. coli communis</i> <i>Staphylococcus aureus</i>	U I U U U	D D D D D
9 B. G.	Markedly improved	<i>B. coli communior</i> <i>Streptococcus hemolyticus</i>	U U	U D
10 C. C.	Markedly improved	<i>Staphylococcus albus</i> <i>Streptococcus viridans</i>	U U	D D
23 A. B.	Moderately improved	<i>B. coli communis</i> <i>Micrococcus catarrhalis</i> <i>Enterococcus indifferens</i> <i>Staphylococcus albus</i> <i>Staphylococcus aureus</i>	U U U U D	U D D D D
24 M. V.	Unimproved	<i>B. coli communis</i> <i>Staphylococcus aureus</i> <i>Staphylococcus albus</i>	U U U	I D D
25 J. S.	Slightly improved	Atypical gram neg. coccus <i>B. alkaligines</i> <i>B. morgani</i> <i>Streptococcus hemolyticus</i>	U D I I	D D U U
26 R. S.	Completely relieved	<i>B. coli communior</i> <i>B. coli communis</i> <i>B. coli communis hem.</i>	D D D	D D D

\*Unchanged. †Diminished. ‡Increased.

TABLE III—CONT'D

PATIENT	CONDITION AT TIME OF RETEST	VACCINES USED IN TESTS AND IN TREATMENT	EARLY LOCAL REACTION	LATE LOCAL REACTION
27 E S	Markedly improved	<i>Staphylococcus albus</i> <i>Staphylococcus albus</i> Hem <i>Streptococcus viridans</i>	D D U	D I D
28 F P	Unimproved	<i>Micrococcus catarrhalis</i> <i>Staphylococcus aureus</i> <i>Streptococcus indefinite</i>	U U U	I D D
29 E P	Markedly improved	<i>B. coli communis</i> <i>B. coli communis</i> hem <i>Enterococcus indifferens</i>	U U U	U U D
30 H W	Completely relieved	<i>B. coli communior</i> <i>B. coli communior</i> hem <i>Enterococcus viridans</i>	U D D	D D D
31 C W	Completely relieved	<i>B. coli communis</i> <i>Streptococcus indifferens</i> <i>Streptococcus indifferens</i>	U D D	D D D
32 E S	Markedly improved	<i>B. coli communis</i> <i>B. coli communis</i> hem <i>Enterococcus hemolyticus</i> <i>Enterococcus viridans</i>	U U U U	U D D D
33 J McC	Completely relieved	<i>B. coli communis</i> <i>B. coli communis</i> hem <i>Streptococcus hemolyticus</i> <i>Streptococcus viridans</i>	I U D D	D D D D
Reactions	Unchanged	Decreased	Increased	
Early local	71 (67.0%)	24 (23.0%)	11 (10.0%)	
Late local	19 (17.9%)	77 (72.6%)	10 (9.5%)	

that section as the representative of one of these features. Reference to Table I will assist the reader in understanding these graphs. There are listed the features or components observed as parts of the late local reaction. These bear the initial letters of their names as symbols in our usual method of recording their presence in reactions.<sup>1</sup> Repetition of a symbol two or more times signifies that the feature represented is two or more times larger than the minimum size. In like manner, in the graphs, the amount of horizontal extension of a block indicates the quantity of a separate feature. The transverse lines drawn between the N-R groups of blocks and those blocks above them indicate a separation of the nodule macule from all other features of the reaction. We have evidence which suggests that the nodule macule portion of the reaction is associated with an immunity mechanism separate from that of bacterial allergy of which the other components are manifestations.<sup>4</sup> The late local reactions seen before treatment and those seen after treatment are recorded in both of the charts, the late local reactions observed during the course of treatment are also recorded in Chart 2. The number of vaccine doses and the total amount of vaccine substance, in millunits (MU),<sup>\*</sup> received by each patient as well as other pertinent data are also recorded.

\*We have used the arbitrary standards of vaccine units and millunits as measures of quantity of bacterial substance in testing and treatment vaccines. The Vaccine unit is 0.01 c.c. of a 1 per cent volumetric suspension of killed bacteria and the millunit is 0.001 of a unit.<sup>4</sup>

TABLE IV

CHANGES IN THE SKIN SENSITIVITY OF TWENTY-FOUR PATIENTS TO VACCINES WHICH WERE NOT USED IN TREATMENT. THE CHANGES ARE EXPRESSED IN TERMS OF THE EARLY AND LATE LOCAL REACTIONS

PATIENT	CONDITION AT THE TIME OF RETEST	VACCINES USED IN TESTS BUT NOT IN TREATMENT	EARLY LOCAL REACTION	LATE LOCAL REACTION
3 B. B.	Markedly improved	<i>B. coli communis</i> <i>Staphylococcus albus</i> <i>Streptococcus viridans</i> <i>Streptococcus viridans</i> <i>Streptococcus viridans</i> <i>Streptococcus hemolyticus</i>	U* U U I‡ U U	D† D U U U U
4 R. P.	Moderately improved	Atypical gram neg. coccus <i>Streptococcus indifferens</i>	U U	D U
5 C. B.	Completely relieved	<i>Micrococcus catarrhalis</i> <i>Streptococcus hemolyticus</i> <i>Streptococcus viridans</i>	U U U	D D D
7 A. W.	Slightly improved	<i>Streptococcus indifferens</i> <i>Streptococcus hemolyticus</i> <i>Micrococcus albus</i>	U U U	D U U
8 A. V.	Markedly improved	<i>Streptococcus viridans</i> <i>B. coli communior</i>	U U	I D
9 B. G.	Markedly improved	<i>Micrococcus catarrhalis</i> <i>Streptococcus indifferens</i> <i>Streptococcus viridans</i>	U U U	U U D
10 C. C.	Markedly improved	<i>Staphylococcus albus</i> <i>Streptococcus indifferens</i>	U U	U D
11 C. R.	Moderately improved	<i>Streptococcus viridans</i> <i>Staphylococcus aureus</i> <i>Staphylococcus albus</i> <i>Staphylococcus albus</i>	I U U D	D I U I
12 A. H.	Completely relieved	<i>Streptococcus viridans</i>	U	U
13 B. S.	Slightly improved	<i>Staphylococcus aureus</i> <i>Streptococcus viridans</i>	I I	I U
15 A. W.	Completely relieved	<i>Staphylococcus aureus</i> <i>Streptococcus hemolyticus</i> <i>Streptococcus viridans</i> <i>Streptococcus viridans</i>	U U U U	D D I I
16 C. M.	Completely relieved	<i>Staphylococcus albus</i> <i>Streptococcus hemolyticus</i> <i>Streptococcus viridans</i> <i>Staphylococcus albus</i>	U D D D	I U I D
17 T. D.	Moderately improved	Chromogenic coccus 6 Chromogenic coccus 6	I U	D D
18 W. S.	Markedly improved	<i>Streptococcus viridans</i>	U	D
22 F. J.	Moderately improved	<i>Streptococcus hemolyticus</i> <i>Streptococcus viridans</i>	I U	U U
24 M. V.	Unimproved	<i>Staphylococcus aureus</i> <i>Streptococcus hemolyticus</i>	U U	U I
25 J. S.	Slightly improved	<i>Staphylococcus albus</i> <i>Staphylococcus albus</i> <i>B. diphtheroid</i>	U D U	U U U

\*Unchanged, †Diminished, ‡Increased.

TABLE IV—CONT'D

PATIENT	CONDITION AT THE TIME OF RETEST	VACCINES USED IN TESTS BUT NOT IN TREATMENT	EARLY LOCAL REACTION	LATE LOCAL REACTION
26 R S	Completely relieved	<i>Enterococcus indifferens</i>	U	U
27 E S	Markedly improved	<i>Staphylococcus albus</i>	D	I
		<i>Chromogenic coccus b</i>	I	I
		<i>Streptococcus indifferens</i>	U	D
		<i>Streptococcus indifferens</i>	U	I
		<i>Streptococcus viridans</i>	U	I
28 F P	Unimproved	<i>Staphylococcus albus</i>	U	D
29 E P	Markedly improved	<i>Enterococcus hemolyticus</i>	U	D
		<i>Enterococcus viridans</i>	U	D
30 H W	Completely relieved	<i>E. coli communis</i>	D	D
31 C W	Completely relieved	<i>Enterococcus viridans</i>	U	D
		Atypical gram neg coccus	U	U
		<i>Staphylococcus albus</i>	D	D
		<i>Streptococcus hemolyticus</i>	U	U
		<i>Streptococcus viridans</i>	U	U
33 J McC	Completely relieved	<i>Streptococcus hemolyticus</i>	U	D
Reactions		Unchanged	Decreased	Increased
Early local		46 (74.2%)	8 (12.9%)	8 (12.9%)
Late local		24 (38.7%)	25 (40.4%)	13 (20.9%)

TABLE V

SUMMARY OF CHANGES NOTED IN SKIN SENSITIVITY OF THIRTY THREE PATIENTS TO THE AUTOGENOUS VACCINES WITH WHICH THEY WERE TREATED

PATIENTS		NO OF VAC CINES	CHANGES IN SKIN SENSITIVITY AS MANIFESTED BY					
NO	CLINICAL RESULT		EARLY LOCAL REACTION			LATE LOCAL REACTION		
			UNCHG	DECF	INCF	UNCHG	DECF	INCF
21	Completely relieved or markedly improved	67	45 67 1%	18 26 9%	4 6 0%	7 10 5%	57 85 1%	3 4 5%
6	Moderately improved	18	12 66 7%	4 22 2%	2 11 1%	4 22 2%	12 66 7%	2 11 1%
6	Slightly improved or un improved	21	14 66 7%	2 9 5%	5 23 8%	8 38 1%	9 42 9%	5 23 8%

TABLE VI

SUMMARY OF CHANGES NOTED IN SKIN SENSITIVITY OF TWENTY FOUR PATIENTS TO SOME OF THE AUTOGENOUS VACCINES WHICH WERE NOT USED IN TREATMENT

PATIENTS		NO OF VAC CINES	CHANGES IN SKIN SENSITIVITY AS MANIFESTED BY					
NO	CLINICAL RESULT		EARLY LOCAL REACTION			LATE LOCAL REACTION		
			UNCHG	DECF	INCF	UNCHG	DECF	INCF
15	Completely relieved or <i>markedly</i> improved	41	32 78.0%	7 14.6%	2 7.4%	31 75.6%	10 48.8%	9 22.0%
4	Moderately improved	10	1 60.0%	1 10.0%	1 10.0%	4 40.0%	1 40.0%	5 20.0%
5	Slightly improved or un improved	11	8 72.7%	1 9.1%	2 18.2%	7 63.6%	2 18.2%	2 18.2%



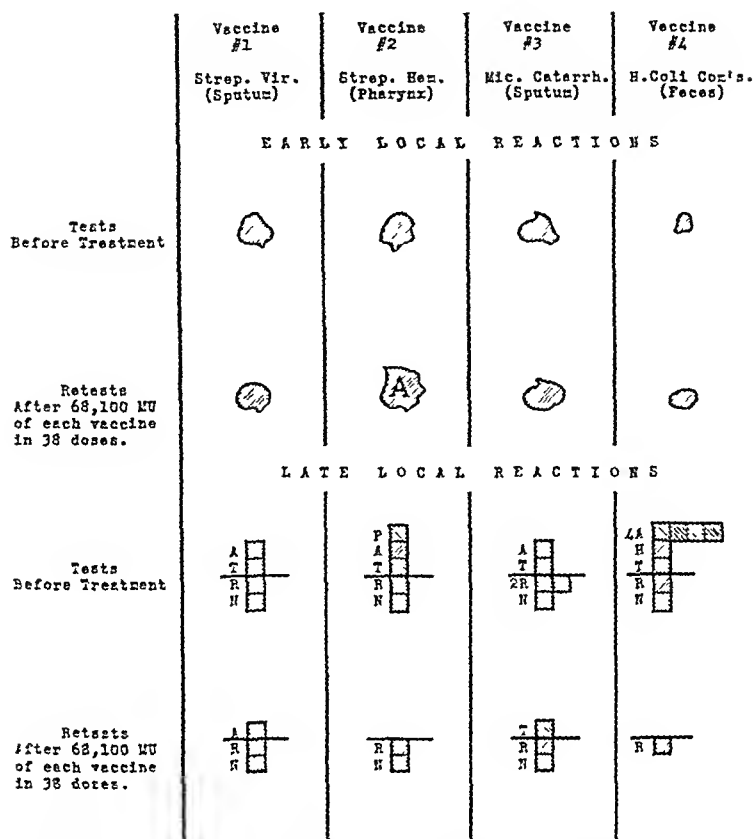


Chart 1.—Graphic representation of early and late local reactions produced by vaccines used in treatment. Patient, A. V. Diagnosis: asthma and urticaria. Duration of treatment: eighteen months. Condition at time of retest: markedly improved.

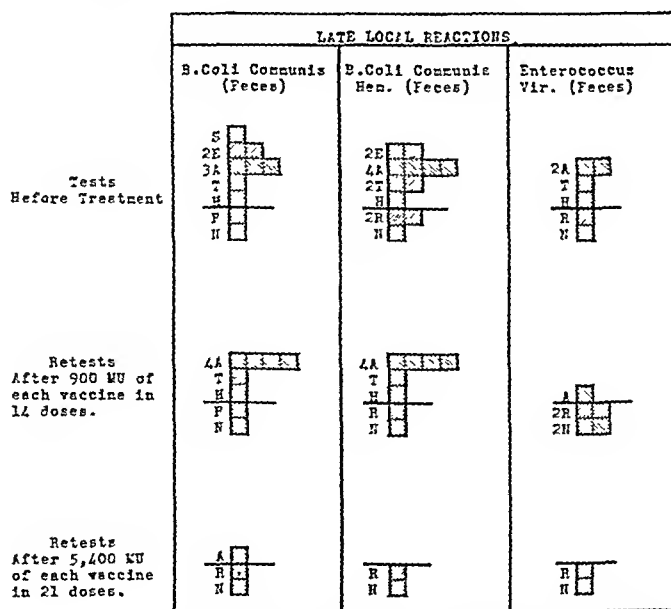


Chart 2.—Graphic representation of late local reactions produced by vaccines used in treatment. Patient, H. W. Diagnosis: mixed type of arthritis. Duration of treatment: twelve months. Condition at end of treatment: completely relieved.

Chart 1 presents the data of a patient who had asthma and urticaria. After eighteen months of treatment with autogenous vaccines she was markedly improved. A total of 61,000 millimits of each of four vaccines had been administered in thirty eight doses. The local reactions following vaccine skin tests with each of these organisms before treatment and those following identical tests after treatment are illustrated. It is seen that there was but little difference between the wheals produced by the several vaccines before treatment and those produced by the same vaccines after treatment. In the case of the late local reactions, on the other hand, regressive changes in skin sensitivity to each of the vaccines were observed.

The patient whose late local reactions are graphically recorded in Chart 2 suffered with chronic arthritis of the mixed type. She received a total of 5,400 millimits of each of three autogenous vaccines in twenty-one doses. The late local reactions following skin tests with each of these vaccines before treatment and those which followed identical tests during and again after treatment are illustrated. At the time of the first retests, namely six months after treatment was begun, the patient was markedly improved. She had become symptom free before the second retests were made. Progressive regression in skin sensitivity to all of the vaccines is seen to have occurred in a manner which paralleled diminution in and final disappearance of the symptoms of arthritis.

#### DISCUSSION

The results of observations in this small series of patients are typical of what has been found in the majority of a large number of patients similarly tested and treated. As we have stated in previous articles<sup>1-5</sup> these changes are largely limited to that form of skin sensitivity which is manifested by the late local vaccine reaction and, usually, are regressive in character. An increase in skin sensitivity to one or more organisms has been seen to occur after treatment with mixed vaccines, but never after treatment with single strain vaccines. Other writers have reported increased skin sensitivity following intracutaneous and subcutaneous vaccine injections.<sup>6</sup> Clawson and Wetherby,<sup>7</sup> in a report on intravenous vaccine therapy in arthritis make the following statement: "The subcutaneous method (of vaccine injection) seems to be contraindicated, since it tends to increase hypersensitiveness, does not bring about a state of desensitization and produces only a low degree of protection." It appears to us that an increase in skin sensitivity after vaccine injections occurs only when too large a dose of killed bacteria or some other factor, such as the use of a mixed vaccine, establishes a reservoir of antigenic material in or beneath the skin. Under these circumstances a lesion results which has the effect of a focus of infection. An increase in bacterial allergy may follow if a series of such injections is given.<sup>8</sup> Treatment injections administered by us, however, using vaccine reactions as guides, are adjusted in bulk and concentration so as to produce a minimum of local reaction. Thus the production of focal lesions and an increase in hypersensitivity have usually been avoided. Retests in patients under treatment with mixed vaccines composed of one organism which produced a large late local reaction after the original

skin tests and of others which produced smaller reactions have, at times, revealed increased skin sensitivity to some of these organisms. At other times, it was seen after retests, that reduction in skin sensitivity was proceeding at a more rapid pace in the case of some of the organisms contained in a mixed vaccine than in the case of others. Further injections of the same organisms, but as single strain vaccines, have appeared to rectify these inequalities. The intense local reaction produced by the organism, in a mixed vaccine, to which the patient is most sensitive seems to localize the less potent organisms within the reaction to such an extent that little desensitizing effect can be produced by them. Such a local reaction may even act in a manner similar to that of a focus of infection from which sensitizing doses of the weaker bacteria repeatedly escape.

We would emphasize two observed facts, viz.: (1) not only did a diminution or disappearance of the delayed type of skin sensitivity (to organisms used in treatment) accompany relief of symptoms but also (2) these two synchronous phenomena proceeded in direct proportion to each other.

Collis and Sheldon<sup>8</sup> noted similar results in children with rheumatic fever who received intravenous injections of streptococcus products. They cite case histories "to show that there is a parallelism between clinical improvement, desensitization and the presence of immune bodies in the serum."

The above facts suggest that the symptoms which were relieved may have been allergic manifestations set up by the particular organisms recovered from the patient and used in treatment. Further evidence to this effect exists in the fact that, in the case of patients who were not relieved by treatment (see Table V), the changes in skin sensitivity to organisms used in treatment were almost exactly the same in degree as those observed in patients on retesting with organisms not used in treatment (see Table IV). In other words, so far as specific desensitization to the particular organisms used in treatment is concerned, no greater changes were produced by treatment in unimproved patients than those which would have occurred without treatment.

Persistence of an undrained focus of infection seems to be the usual cause of failure of autogenous vaccine therapy to be followed by improvement of symptoms and by regressive changes in skin sensitivity. Our experience has encouraged us, in these cases, to resume vaccine therapy after such a focus has been found and eradicated.

#### SUMMARY AND CONCLUSIONS

Early and late local skin reactions following intracutaneous injection of autogenous vaccines are described and defined. Attention is called to certain variations in the reactions which occur when patients are retested during or after courses of vaccine treatment. Data taken from the case histories of thirty-three patients tested and treated with autogenous vaccines are here collected and analyzed in order to demonstrate the following conclusions, namely:

1. Regardless of the degree of clinical improvement following treatment, the early vaccine skin reaction remained relatively unchanged.

2 Regression of the late vaccine skin reaction occurred in the majority of successfully treated patients and failed to occur in most of those receiving no benefit from vaccine treatment

3 The degree of regression in intensity of the late vaccine skin reaction was in direct proportion to the degree of improvement in the clinical condition under treatment

4 Vaccine skin reactions in addition to being useful guides in the selection of vaccine for treatment are indicators of the progress of desensitization in vaccine treated patients

5 Concurrent decrease in the late local vaccine reaction and in the patient's symptoms during vaccine treatment appear to point to a relationship between those symptoms and a specific sensitivity to the organisms used

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## THE NORMAL RANGE OF THE LEUCOCYTE COUNT DETERMINED WEEKLY OVER AN EXTENDED PERIOD\*

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THIS study was undertaken to determine the range of the leucocyte count taken weekly in normal subjects under standard conditions. Although the project was an investigation in chronic rheumatic heart disease, it seemed necessary to digress in this direction to decide if possible a normal range, so that the findings in the rheumatic group might be properly correlated.

A perusal of some of the literature revealed many valuable observations as well as many conflicting ones. In some instances investigators have taken isolated counts on large groups, some many counts on small groups, and then again others have noted the influence of many phenomena, such as heat, cold, rest, exercise, and digestion. In their review Sabin, Cunningham, Doan, and Kindwall<sup>1</sup> mention the findings of Galambos, Turk, and Torday, who observed the normal range to be 3,500 to 12,500, 5,000 to 10,000, and 3,130 to 9,800, respectively. Sabin et al.<sup>1</sup> in their study of six normal subjects noted an hourly rhythm of the leucocyte count with a progressive increase in the afternoon, the lowest level being in the morning, whether or not food had been taken. The variation was from 5,000 to 10,000, a ratio of 1:2. The upper limit was 13,000 in one case. Smith and McDowell<sup>2</sup> corroborated these findings and in addition observed no effect of menstruation on the count. Two cases were studied. According to these authors the curves of the daily rhythm had a characteristic pattern for each person. Swift, Miller, and Boots<sup>3</sup> found 8,000-9,000 the upper limit of normal in a group of nonrheumatic persons and patients who had completely recovered from the infection. Their studies were made between eleven and twelve in the morning or three and four in the afternoon. Garrey and Butler<sup>4</sup> determined that at rest the basal count was 5,000 to 6,000, the physical level being 60 per cent to 100 per cent above the basal level. Their observations are in accord with those of Sabin et al.<sup>1</sup> and Smith and McDowell<sup>2</sup> with regard to the fact that there is no digestive leucocytosis.

The unusually wide range of the alleged normal and the varied methods of observation made it necessary to set up a standard method of study. The leucocyte curves of Sabin et al.<sup>1</sup> and those of Smith and McDowell<sup>2</sup> clearly demonstrated that the uniformly low level was the forenoon. The time

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chosen, therefore, was between 10 30 A M and 12 M on Thursday of each week when possible. No unusual physical exertion was engaged in and there was no exposure of the hands to extreme heat or cold prior to the procedure, in order to eliminate some of the usual variants. The group consisted of 11

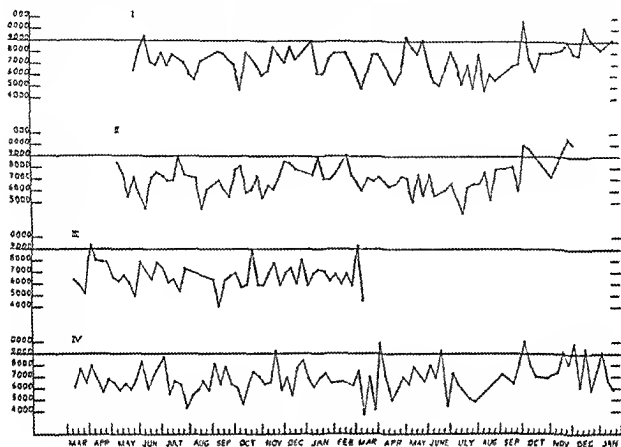


Chart 1

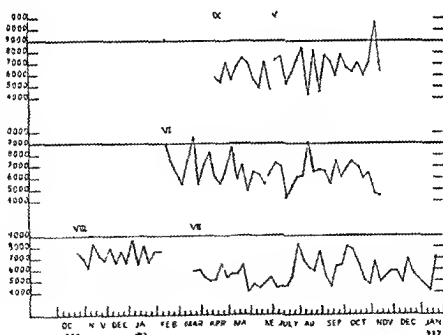


Chart 2

persons between the ages of seventeen and fifty. 7 females and 4 males studied for a period from eleven weeks to ninety eight weeks. The lowest number of counts was 11 the highest 85.

The curves were so strikingly similar in 9 individuals that 2 were excluded as being abnormal but are also reported. Charts 1 and 2 represent

the leucocyte curves of the nine cases. The ordinates indicate the date of each count and the abscissas the leucocyte count. (Charts 1 and 2.) All the counts were below 11,000 in the upper limit; 435 counts were made, 94 per cent of which were below 9,000. This finding is in agreement with that of Swift, Miller and Boots.<sup>3</sup> All the counts with one exception were above 4,000 in the lower limit. For practical purposes, therefore, the normal range of the leucocyte count is between 4,000 and 9,000. The ratio of 1:2 is similar to that obtained by Sabin et al.<sup>1</sup> and Smith and McDowell<sup>2</sup> in their series.

The curves are practically identical with those of the forenoon of these authors regardless of the fact that the latter were serial determinations on the same day. Furthermore, a careful examination of the morning portions of their curves showed the upper limit to be below 9,000 and the lower limit above 4,000.

It will also be noted in Charts 1 and 2 that season, sex, and age within limits of the group had no influence on the counts. The curves did not show an individual pattern but appeared to fall within 2 groups, 7 in one and 2

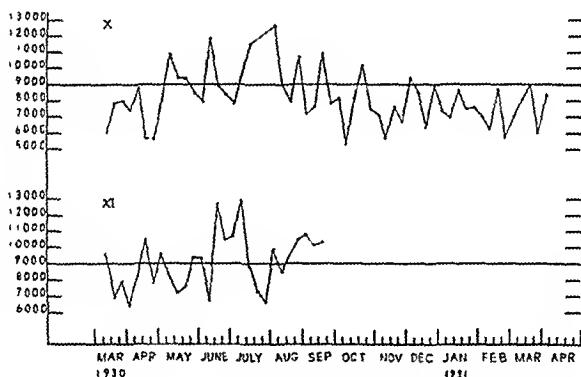


Chart 3.

(Cases 8 and 9) in the other. The latter 2 did not have as wide a range as the former group, the ratio being less than 1:2. This appeared to be the difference between Subject A and Subject B in Smith and McDowell's series.<sup>2</sup> If these authors had studied more than 2 cases perhaps a similar grouping might have been found rather than individual patterns.

Table I shows the number of counts taken on each individual and the percentage below 9,000.

Table II shows the range of counts including the average. It will be noted that the highest average count was 7,300 and that the two abnormal were 8,100 and 9,100, respectively.

Chart 3 shows the leucocyte curves which were considered abnormal. Case 10 has two phases, the first abnormal and the second similar to the curves in the normal group. Case 11 in no way resembles any of the other curves.

There was no recognized physical basis to account for the two abnormal curves. These individuals were in a more emotional state than the others:

the observations of Garvey and Butler<sup>4</sup> who noted leucocytosis in psychologic states might explain these deviations. In addition they were subject to more upper respiratory infections than the rest.

TABLE I

	CASE	TOTAL NUMBER OF COUNTS	PERCENTAGE BELOW 9,000
Normal	1	74	90
	2	70	89
	3	48	96
	4	85	90
	5	20	95
	6	79	94
	7	41	100
	8	16	100
	9	11	100
Abnormal	10	52	77
	11	27	48

TABLE II

	CASE	LOWEST	HIGHEST	AVERAGE
Normal	1	4,700	10,700	7,300
	2	4,200	10,600	7,100
	3	4,000	9,300	6,700
	4	3,700	10,000	6,900
	5	4,300	10,700	7,100
	6	4,300	9,600	6,600
	7	4,000	8,300	5,600
	8	6,200	8,600	7,300
	9	4,800	7,500	6,100
Abnormal	10	5,300	12,600	8,100
	11	6,400	12,900	9,100

# SUMMARY

1 The leucocyte counts were determined weekly in eleven normal subjects between the ages of seventeen and fifty. They were studied for a period of eleven to ninety-eight weeks under standard conditions. Counts were taken between 10:30 A. M. and 12 M. once a week, eliminating exposure to heat and cold and avoiding exercise, factors which influence the number of white blood cells.

2 The leucocyte curves of nine persons were so similar that two were excluded as abnormal but are also reported.

3 The curves presented no individual patterns. There appeared, however, to be a possible grouping with seven in one and two in the other.

4 The normal range of the count was 4,000 to 9,000.

5 Sex, season, and age within limits of the group had no influence on the leucocyte count.

# CONCLUSION

Under standard conditions the normal range of the leucocyte count is 4,000 to 9,000.

Many thanks are due Doctors Alfred E. Cohn and Homer F. Swift for their most valuable advice and constructive criticism during the period of study; to Mr. Harry Hopkins for his assistance in arranging the budget; and to Doctor Edward Holtz who assisted me during the study.



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## THE SIGNIFICANCE OF SERUM INORGANIC SULPHATE CONCENTRATIONS IN BRIGHT'S DISEASE<sup>2</sup>

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THE physiologic and clinical significance of serum inorganic sulphate concentrations has until recently received little attention. Denis,<sup>1</sup> in 1921, reported a nephelometric method for the determination of inorganic sulphate in blood. She found from 0.5 to 1.0 mg. of sulphate sulphur in the serum of normal persons, with much larger concentrations in the serum of patients with terminal Bright's disease. The well-known technical difficulties inherent in nephelometric procedures have prompted other investigators to develop new methods for serum sulphate. Most of these methods utilize a trichloroacetic acid filtrate from serum and differ from each other merely in the scheme for analyzing the benzidine sulphate precipitated in this filtrate. Though there is a comparatively wide variation in the values obtained by these methods, the results of most of them agree with each other and with those of Denis in two essential features—first, that normal serum inorganic sulphate concentration is of the order of 1 mg. S per 100 c.c., and second, that there is a marked rise in this value as renal inefficiency progresses to uremia in Bright's disease.

Wakefield, Power, and Keith<sup>2</sup> have drawn further conclusions. Reporting on the use of a new oxidimetric method for analyzing benzidine sulphate, they compared serum sulphate concentrations with the results of standard tests for renal function in a large number of individuals. Their cases included normal persons, those with essential hypertension, those with early Bright's disease with and without edema, and those with terminal Bright's disease. These authors have found that in uremia serum sulphate concentration rises along with urea, reaching values of more than 30 times that of normal. In addition, they reported that many patients with hypertension or with early glomerulonephritis, who as yet showed no deviation from the

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normal in most of the common tests for renal efficiency, including that of urea clearance, already showed elevations of serum inorganic sulphate. These authors submit the determination of serum sulphate as a test of early renal inefficiency similar to and as valuable as the concentration test, the phenol-sulphonephthalein test, and the urea clearance estimation.

These results are obviously of great importance if corroborated. Aside from the theoretical physiologic significance of such early rises in serum inorganic sulphate concentration, the practical value of being able to gauge early renal inefficiency by a comparatively simple determination of a blood constituent would be very much appreciated by clinicians. The present investigation was undertaken primarily for the purpose of such a corroboration. But the study has been broadened to include the rôle of serum sulphate in more advanced Bright's disease, especially its effect upon the production of acidosis in uremia.

#### METHODS

A critical survey was first attempted of the recent methods for determining serum sulphate. It was soon discovered that those methods, including that of Power and Wakefield,<sup>3</sup> which depend upon the precipitation of benzidine sulphate from a trichloroacetic acid filtrate of serum by the addition of benzidine in acetone, are subject to serious errors because the precipitate obtained is contaminated with other substances particularly benzidine phosphate. The values obtained by such methods may be too high by about 35 per cent in normal serum and by as much as 300 per cent in serum from uremic patients.

A new method has therefore been developed in this laboratory by Hoffman and Cardon,<sup>4</sup> which attempts to avoid these errors and which gives equally consistent results with both normal serum and that from uremic patients. In this method, a protein free, phosphate free filtrate from serum is obtained by coagulating the diluted serum by heat in the presence of ferric chloride, ammonium acetate, and ammonium hydroxide, as in the senior author's<sup>5</sup> method for serum total base. In this water clear filtrate, sulphate can be precipitated in the usual manner by the addition of benzidine chloride and acetone. Pure satiny crystals of benzidine sulphate are obtained, which, after washing three times with acetone in a specially designed centrifuge tube, are analyzed by oxidation with  $\text{KMnO}_4$ , as in Kramer and Tisdall's<sup>6</sup> method for serum potassium.

The average concentration of serum inorganic sulphate by this method in forty-two normal persons was 0.78 mg S per 100 cc with a standard deviation of +0.16 mg (see Fig. 1). The maximal normal value found was 1.09 mg S per 100 cc.

In order to ascertain the rôle of serum sulphate in Bright's disease, a study was made of sixty patients. In these patients, simultaneous determinations were made of serum sulphate, blood urea nitrogen, and the urea clearance. In addition, in those cases of markedly elevated serum sulphate, com-

plete electrolyte balance studies were attempted for the purpose of estimating the part played by sulphate in the production of the acidosis so characteristic of uremia.

The chemical methods employed were the following: urea nitrogen, Van Slyke and Cullen;<sup>7</sup> urea clearance, Moeller, McIntosh and Van Slyke;<sup>8</sup> total base, Hoffman;<sup>5</sup> chloride, Van Slyke and Sendroy;<sup>9</sup> phosphate, Fiske and Subbarow;<sup>10</sup> protein, macro Kjeldahl; carbon dioxide, Van Slyke and Cullen;<sup>11</sup> serum sulphate, Hoffman and Cardon.<sup>4</sup>

The estimation of base combined with phosphate, protein, and bicarbonate was made from data given by Peters and Van Slyke,<sup>12</sup> assuming a serum pH of 7.35. This of course involves an error; but the extent of the error for the total anions probably does not exceed 2 milliequivalents. For the purpose of electrolyte balance studies, an error of such magnitude is insignificant.

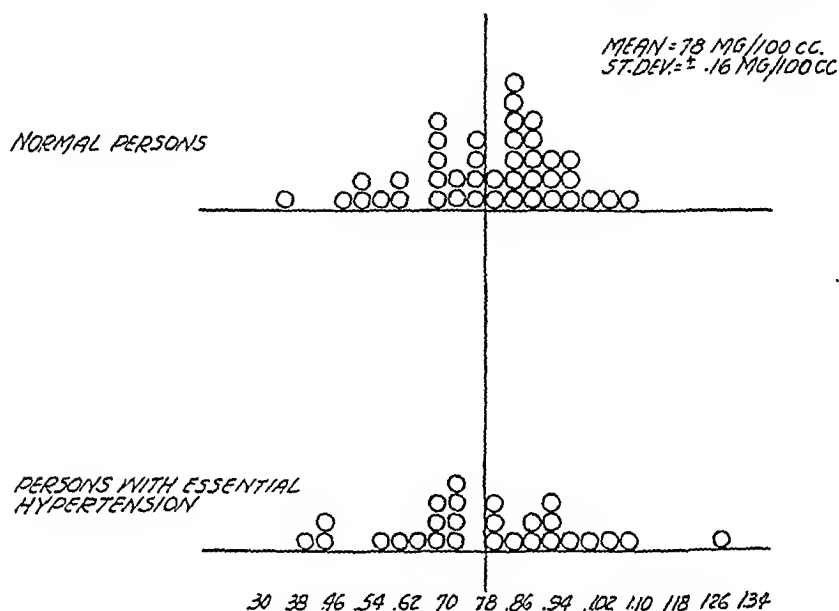


Fig. 1.—Frequency chart showing the range of serum inorganic sulphate in normal adults and in patients with essential hypertension.

Undetermined organic acid was estimated by subtracting the total of the determined anions from the total base. In normal serum, these undetermined acids ranged from 3 to 6 millicivalents per liter.

## RESULTS

In Tables I, II and III are presented the results of the chemical studies made on all sixty patients. The data from twenty-seven patients with hypertension with comparatively little renal involvement are shown in Table I. In Table II a miscellaneous group of cases of Bright's disease are presented. Table III includes the electrolyte balance studies made on those patients who were in or near uremia. With the exception of the last two cases, in which cardiac failure was the most prominent finding, the cases in Table III are given in the apparent order of severity of uremic symptoms.

TABLE I

THE RELATIONSHIP BETWEEN SERUM INORGANIC SULPHATE, BLOOD UREA, AND UREA CLEARANCE IN CASES OF ESSENTIAL HYPERTENSION

CASE	SEX AND AGE	BLOOD UREA MG N PER 100 CC	SERUM SULPHATE MG S PER 100 CC	UREA CLEARANCE PER CENT OF NORMAL
1	F 24	97	0.85	67
2	M 21	19.9	0.92	42
4	F 54	17.4	0.89	58
5	M 28	17	0.78	111
6	F 42	12.9	0.88	58
7	M 52	19.4	0.93	96
11	F 46	24.1	0.71	70
12	F 48	15.4	0.81	42
17	M 37	15.1	1.26	71
19	M 55	15.1	0.72	57
20	F 38	19.2	0.68	57
22	F 38	97	0.45	74
23	F 36	9.0	0.63	35
25	M 50	23.2	1.06	69
26	M 35	14.4	1.02	74
28	F 27	97	0.73	51
29	F 26	10.7	0.55	42
30	M 55	13.0	0.98	80
36	M 23	21.6	0.91	08
39	M 67	12.5	0.39	48
40	M 54	19.0	0.80	34
44	M 56	10.0	0.43	98
45	F 42	13.6	0.60	53
46	M 45	0.4	0.68	52
47	F 62	11.8	0.94	45
50	F 40	22.7	0.73	35
55	F 40	11.0	0.67	44

TABLE II

THE RELATIONSHIP BETWEEN SERUM SULPHATE, BLOOD UREA AND UREA CLEARANCE IN MISCELLANEOUS TYPES OF BRIGHT'S DISEASE

CASE	SEX AND AGE	BLOOD UREA MG N PER 100 CC	SERUM SULPHATE MG S PER 100 CC	UREA CLEARANCE PER CENT OF NORMAL	DIAGNOSIS
3	M 42	37.2	2.87	13	Malignant nephrosclerosis
8	M 53	176.1	6.60	4	Malignant nephrosclerosis
10	F 36	26.4	0.77	25	Chronic glomerulonephritis
18	M 40	33.2	0.73	30	Chronic glomerulonephritis
21	M 74	22.5	0.49	24	Arteriosclerosis
31	M 55	19.0	1.42	31	Cardiac decompensation
32	M 38	41.9	1.75	20	Cardiac decompensation
33	M 40	39.6	2.44	27	Cardiac decompensation
37	M 27	22.5	1.17	60	Amyloid nephrosis
38	M 55	169.0	7.90	-	Nephrosclerosis, coma
41	M 43	94.0	3.39	5	Malignant nephrosclerosis
43	M 48	35.4	0.89	43	Cardiac decompensation
49	M 21	25.4	1.09	50	Chronic glomerulonephritis with edema
51	M 31	76.2	1.50	16	Chronic glomerulonephritis with edema
60	M 34	44.0	2.70	22	Chronic glomerulonephritis with edema

TABLE III  
CHEMICAL STUDIES OF THE SERUM FROM PATIENTS WITH TERMINAL BRIGHT'S DISEASE

CASE	SEX AND AGE	BLOOD UREA	UREA CLEARANCE	SERUM PHATE	TOTAL BASE	CHLO- RIDE	HCO <sub>3</sub>	PROTEIN (B PR)	PHOS PATE	UNDETHER- MINED ACID	SUL- PATE	DIAGNOSIS
		MG. N PER 100	PER CENT OF NORMAL	MG. S PER 100 G.C.	M. EQ. PER LITER	M. EQ. PER LITER	M. EQ. PER LITER	M. EQ. PER LITER	M. EQ. PER LITER	M. EQ. PER LITER	M. EQ. PER LITER	
AU- THORS'	NORMALS	8.23	75-120	0.4-1.1	153-158	100-106	25-28	15-17	2.3	3.6	0.3-0.7	
16	M 43	40.8	27	0.95	154.2	106.2	25.5	17.8	2.6	1.2	0.6	Nephrosclerosis, not in uremia
13	M 58	102.2	7	4.30	153.2	105.7	22.8	16.4	3.2	2.4	2.7	Malignant nephrosclerosis; beginning uremia
52	M 32	118.8	7	4.83	153.1	103.0	24.4	14.3	4.1	4.3	3.0	Chronic glomerulonephritis; beginning uremia
15	M 24	82.9	11	2.44	152.0	106.0	22.8	14.1	3.0	4.6	1.5	Chronic glomerulonephritis; beginning uremia
53	M 51	132.1	4	3.21	151.5	110.0	13.5	15.4	3.9	6.6	2.1	Malignant nephrosclerosis; beginning uremia
27	M 41	158.1	--	8.60	149.0	108.9	12.8	13.2	5.9	2.8	5.4	Malignant nephrosclerosis; uremia
48	F 42	60.0	6	1.62	155.0	110.0	25.0	15.1	2.8	1.1	1.0	Nephrolithiasis with hydronephrosis
54	F 43	115.1	--	3.00	138.6	102.2	8.9	15.9	4.7	5.0	1.9	Same patient in uremia 3 months later
34	M 42	162.5	4	6.23	146.7	99.2	17.9	15.0	5.6	5.1	3.9	Malignant nephrosclerosis; uremia
14	M 25	172.6	4	11.10	144.4	94.0	17.9	16.1	7.1	2.1	6.9	Chronic glomerulonephritis; uremia
56	M 17	260.1	--	12.74	145.0	100.8	9.0	11.6	9.3	6.3	8.0	Chronic glomerulonephritis; uremia
35	M 41	245.1	--	12.26	141.8	90.4	15.1	14.5	9.3	4.8	7.7	Chronic glomerulonephritis; uremia
58	F 46	157.1	--	7.71	135.8	87.7	15.3	12.8	7.1	8.1	4.8	Nephrosclerosis; uremic coma
24	M 38	217.0	--	7.49	146.1	95.5	7.7	19.7	7.5	5.0	4.7	Malignant nephrosclerosis; uremic coma
42	M 39	186.0	--	8.34	142.0	93.2	15.3	16.4	7.3	4.6	5.2	Malignant nephrosclerosis; uremic coma
59	F 31	186.2	--	8.82	132.6	86.3	13.7	16.0	9.1	8.0	5.5	Malignant nephrosclerosis; uremic coma
9	M 55	111.0	--	2.26	156.0	112.1	20.0	16.7	2.2	3.6	1.4	Cardiac decompensation; coma
57	M 41	70.0	--	0.66	149.2	113.5	19.1	9.5	2.4	4.3	0.4	Cardiac decompensation; coma

The patients with essential hypertension showed in almost all cases normal serum sulphate as well as urea. This was true even in those cases in which urea clearance was already below the minimal normal value of 75 per cent. Even those patients who already showed elevations of blood urea gave most often normal sulphate values. In the miscellaneous group of patients it was found too that serum sulphate was usually within normal limits, and that not until urea nitrogen was distinctly elevated above normal was there a consistent rise in sulphate. There are several exceptions (Cases 17, 31, 37). In these, sulphate was elevated even though blood urea nitrogen was still below the maximal normal of 23 mg per 100 cc. Two of these cases (31 and 37) had the complicating factor of edema.

These findings are perhaps better visualized when expressed graphically. In Fig. 1, the distribution of serum sulphate in twenty seven cases of hyper

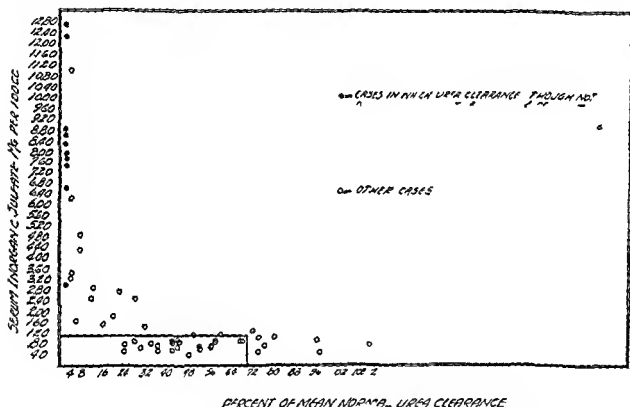


Fig. 2.—Relationship between serum inorganic sulphate and urea clearance in patients with hypertension or Bright's disease. The points enclosed in the rectangle represent sulphate values found within normal limits when the urea clearance already showed renal function less than the normal minimum.

tension is compared with that of the forty two normal persons studied by Hoffman and Candon.<sup>4</sup> The distribution, with the exception of one value, is obviously similar.

In Fig. 2, serum sulphate is plotted against the urea clearance in all the cases studied. In some of the uremic patients, it was impossible to determine urea clearances. In such patients with blood urea nitrogen of 150 mg per 100 cc or higher, it has been found by Van Slyke and others<sup>13</sup> and by other investigators including ourselves that the urea clearance was most often below 4 per cent. Such an assumption has therefore been made in these cases. The plotted values in this chart have a logarithmic distribution, sulphate tending to remain constant until urea clearance had dropped to low values, then rising rapidly as urea clearance sinks still further. It can be seen that

twenty-four cases still showed normal sulphate values when the urea clearance had already dropped below the minimal normal.

The logarithmic distribution of values in Fig. 2 is similar to that found by Van Slyke and his coworkers<sup>12</sup> when they plotted blood urea nitrogen against urea clearance. Such a similarity suggests a correlation between the levels of serum sulphate and urea. Fig. 3 illustrates this correlation more directly. Serum sulphate is seen to remain around normal until urea N rises to about 35 mg. per 100 c.c. Then the two values rise together, so that in uremia both are from 10 to 15 times as high as normal.

In Table III, sulphate, like other serum anions, is expressed in terms of milliequivalents of base neutralized. In this way, the rôle of sulphate in producing a lowering of the alkali reserve (as expressed by  $\text{HCO}_3$ ) can be estimated. In uremia, sulphate is seen to have risen to as high as 8 milliequivalents per liter, which per se would contribute considerably to the

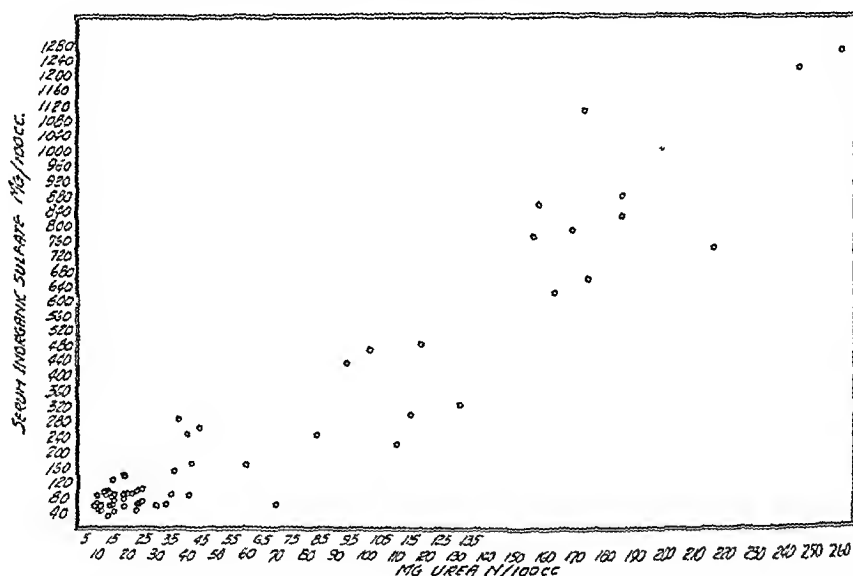


Fig. 3.—Relationship between serum inorganic sulphate and blood urea nitrogen in patients with hypertension or Bright's disease.

production of acidosis. In all cases of markedly elevated sulphate, phosphate had similarly risen. The rise in the two anions accounts for most of the drop in bicarbonate. In a number of cases, however, the lowered bicarbonate was found partly due to a lowering of total base without an equivalently great reduction of chloride. In fact, in several cases (53, 27, 57) serum total base was low while chloride was even higher than normal. Whether this condition is a genuine phase of uremia or whether it is due to the particular clinical management of these patients could not be determined. In severe uremia, particularly after gastrointestinal symptoms had become prominent, serum total base and chloride were both correspondingly low, and the diminished alkali reserve could be entirely accounted for by the rise in sulphate and phosphate. It should be further pointed out that serum sulphate was highest when serum total base was very low.

## COMMENT

Sulphate, like urea, is a metabolic waste product eliminated from the body chiefly by way of the kidneys. One should therefore expect to find the level of serum sulphate related to the degree of renal efficiency. The results obtained here add confirmation to the already ample evidence of this relationship as reported by Demis,<sup>1</sup> Meyer-Bisch,<sup>14</sup> Loeb and Benedict,<sup>15</sup> Cuthbertson and Tompsett,<sup>16</sup> Wakefield and others,<sup>2</sup> and Macy.<sup>17</sup> When the renal function is greatly impaired, sulphate is retained in the blood.

On the other hand it is hardly to be expected that sulphate retention should occur when the compensating power of the kidneys and circulation is still good, as expressed by normal concentration tests, normal phenol sulphonephthalein excretion, or by normal urea clearance. The present investigation could find no confirmation of the claims of Wakefield and his coworkers of such an early rise in sulphate. On the contrary, many of the hypertension cases reported here showed a lowering of urea clearance to about 50 per cent of the mean normal, without any other indication of renal involvement. In all these cases with one exception, sulphate was within normal limits. In several cases too, when urea was already distinctly elevated, serum sulphate was still within normal limits. It seems therefore, that serum sulphate concentration cannot be used as a test for early renal inefficiency.

The roughly parallel rise of both sulphate and urea in advanced Bright's disease, as shown in Fig. 3 has already been mentioned. Neither Demis nor Wakefield could recognize any distinct correlation between urea and sulphate retentions. But Cuthbertson and Tompsett<sup>16</sup> and Loeb and Benedict<sup>15</sup> did affirm such a relationship. Fig. 3 undoubtedly shows a great deal of scatter and the number of cases is probably too small to make a quantitative estimation of correlation. But it is quite apparent from this chart that when renal efficiency is markedly impaired serum sulphate rises with a rapidity similar to that of urea.

The cause of the acidosis in uremia has been given considerable attention by investigators of Bright's disease. This question is thoroughly discussed by Peters and Van Slyke<sup>18</sup> and by Peters.<sup>19</sup> The primary disturbance is probably the great loss of fixed base in the urine produced by diuresis and by the inability of the damaged kidneys to convert a sufficient amount of urea into ammonium for the purpose of neutralizing excreted acids. This loss of base is reflected internally first as dehydration and second as a lowering of the serum total base concentration. All the uremic patients in the present group showed this phenomenon. However, only in the absence of a corresponding loss of chloride does this loss of base produce a considerable lowering of the alkali reserve. Cases 15, 27, 53, 54, 56 and 57 illustrate the diminution of alkali reserve caused in part by the unbalanced loss of total base and chloride. But in other cases, it is chiefly the retention of sulphate and phosphate that is responsible for the lowered bicarbonate. The relatively undisturbed level of undetermined acids shown in Table III adds confirmation



to the belief that in uremia there is little retention of unknown organic acids, unless a superimposed cardiac failure has allowed an accumulation of lactic acid, as pointed out by Meakins and Long.<sup>19</sup>

These findings are essentially the same as those recently reported by Atchley and Benedict,<sup>20</sup> by Briggs,<sup>21</sup> and by Greene, Wakefield, Power, and Keith,<sup>22</sup> who used other methods for the determination of serum total base and serum inorganic sulphate. However, the extent of the sulphate retention reported here is much less than that found by these authors. The maximum serum sulphate reported by Atchley and Benedict was 19.5 m.eq. per liter, by Briggs, 9.2 m.eq. per liter, by Greene, Wakefield, Power, and Keith, 16.2 m.eq. per liter, whereas the maximum found by the present sulphate method was 8.0 m.eq. per liter. In the paper by Wakefield, Power, and Keith,<sup>2</sup> where no electrolyte studies were made, values of 20 m.eq. or more per liter are suggested. Such high values are improbable, as Peters<sup>18</sup> has pointed out. The rise in serum sulphate, then, is an important factor in the production of nephritic acidosis, but the rôle it plays is probably less significant than that assigned to it by recent investigators.

In spite of the probability that the values for serum sulphate obtained by Wakefield and his coworkers are too high, one cannot entirely dismiss their findings of higher than normal sulphate in many early cases of Bright's disease. Many of their cases which showed this early rise had the complicating factor of edema. Several of our cases, too, of edema of either nephrotic or cardiac type showed an unexpected elevation of serum sulphate (Cases 31, 33, 37, and 60). In such cases of edema, chloride is at times also found higher than normal, even when serum total base is normal or lower than normal.<sup>23</sup> These findings suggest that factors other than renal efficiency may be concerned with the excretion of sulphate. Such a possibility is further enhanced by the work of Macy,<sup>17</sup> who showed that urinary sulphate excretion in normal persons varies markedly throughout the day even though serum sulphate concentration changes comparatively little. Denis and others,<sup>24</sup> too, found that diuretic measures can produce a rapid diminution in serum sulphate. The amount of sulphate excreted, even with normal kidneys, may depend upon the amount of base simultaneously available for excretion. If this is so, one can explain not only the high sulphate in cases of edema but also the apparent lack of correlation between urea and sulphate retentions in some advanced cases of Bright's disease. In order to throw more light on this subject, the authors are at present studying sulphate clearances under varying conditions of base excretion.

#### SUMMARY

A new method for the determination of serum inorganic sulphate has been developed, which avoids the errors of previous methods and which gives equally consistent results both with normal serum and that from pathologic cases. The average concentration of serum sulphate in forty-two normal persons was 0.78 mg. S per 100 c.c. with a standard deviation of  $\pm 0.16$  mg.

Sixty patients with hypertension or Bright's disease were studied for the relationship of serum sulphate to blood urea and urea clearance.

In twenty seven persons with essential hypertension, serum sulphate had a distribution similar to that of normal persons

There was no evidence that a rise of serum sulphate was often the first indication of renal inefficiency. On the contrary, in twenty four out of sixty persons studied serum sulphate was still normal when urea clearance was already below the minimal normal level. In some of these patients, blood urea was already distinctly elevated. Serum sulphate determinations are therefore of little help in determining early renal inefficiency.

In patients with terminal Bright's disease, serum sulphate rose roughly parallel to blood urea, and reached values of 12 mg S per 100 cc or 8 miliequivalents per liter.

An analysis of the electrolyte balance in eighteen cases of uremia showed that the rise of serum sulphate, like that of phosphate, played a considerable rôle in the production of uremic acidosis.

These results are discussed and the suggestion is made that factors other than the degree of renal excretory efficiency may also be concerned with the level of serum inorganic sulphate.

The patients studied in this investigation were from the dispensary of The Chicago Medical School and from the wards of the Cook County Hospital. The authors wish to express their appreciation of the cooperation of the staff of the Cook County Hospital.

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## WATER RETENTION IN OBESITY AS DETERMINED BY THE VOLHARD DILUTION AND McCLURE-ALDRICH TESTS\*

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THERE is a growing belief in this country that obesity is purely alimentary in nature. The champion of this school of thought is Newburgh and his associates.<sup>1</sup> In the continental clinics the problem of obesity is looked upon as of dual character—exogenous and endogenous or constitutional. In practice it is quite difficult to know where exogenous obesity ends and endogenous begins, and in treating the two types of cases very little difference is found in their reaction to the dietary regimen. The purely mechanistic conception of Newburgh, however, does not fully account for certain well-known clinical observations, such as the accumulations of fat limited to certain localized areas of the body in some types of obesity; for example, in the eunuchoid type of individual, fat tends to accumulate in the gluteal region, hips, breasts, and mons pubis. Such an individual may be made at times to lose weight on a restricted diet, yet in the locations where fat accumulates most, the fat is likely to remain. The girdle obesity of pituitary disease and of Dercum's disease, in which fat accumulates in isolated masses in places subject to pressure, while the face and extremities remain uninvolved, would strongly suggest that besides energy intake, other factors, particularly endocrine, are involved.<sup>2</sup> This contention is borne out by the experience of Skoptzi, who developed suprapubic and trochanteric pads of fat after castration, as well as by the frequency with which obesity follows childbirth and menopause. The animal breeder has known for a long time that by means of castration he may readily fatten his stock. The rôle that the endocrine glands play in regulating energy balance and tissue activity is well recognized, but the manner in which a dysfunction of one or another of these glands affects this regulation is not as yet clear. It is interesting

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in this connection to call attention to the observations of Wade H Brown in regard to obesity in rabbits, which he believes to be of hereditary and endocrine origin rather than dietary in character.<sup>3</sup>

The origin of obesity will be better understood when a solution is offered to explain how normal weight is maintained and to explain further why the obese do not continue to gain in weight indefinitely but reach a level of stability for long periods of time. It was Van Noorden's belief that economies in the total metabolism of the obese were conditioned by a hypothyroid state.<sup>4</sup> This he assumed to be evidenced by a low basal metabolic rate. As a matter of fact, the basal metabolic rate is so irregular and inconstant in obesity as to be of no great moment in elucidating the pathogenesis of the disease. In fifty-two cases of obesity in which the patients were under observation at the Duetless Gland Clinic of Temple University Medical School from April, 1931 to April, 1933 only seventeen had a perceptibly low basal metabolic rate. Three patients had unusually high metabolic rates. Two of these, with obesity of cerebral origin, had rates of plus 30 per cent, and plus 37 per cent, respectively, while the third patient, who later developed typical thyrotoxicosis, had a rate of plus 47 per cent. Twenty-nine patients showed basal metabolic rates within the range of normality (see Table I). The extremely low basal rate that is occasionally observed in obesity is not characteristic of the disease. It is well known that a decreased basal metabolism is found in undernutrition, in pituitary emaciation (Simmond's disease), and in advanced Addison's disease. Obesity is not necessarily a symptom of hypothyroidism, at the present time we have three patients who are underweight rather than overweight, and who present otherwise typical symptoms of hypothyroidism with metabolic rates varying between minus 18 per cent and minus 22 per cent.

TABLE I

BASAL METABOLIC RATE IN OBESITY TOTAL NUMBER OF CASES, 52

	NUMBER OF CASES
Below 20%	8
-11% to -19%	9
-1% to -10%	11
Normal	5
+1% to +10%	7
Over +10%	6

Strang and Evans<sup>5</sup> have recently pointed out that when basal metabolic rates are estimated on the basis of active tissue mass rather than on surface area it will be found that metabolism in obesity proceeds on a high rather than on a low level.

*Specific Dynamic Action of Food*—That there are isolated obese patients who are refractory to the specific dynamic action of food is well attested to by the work of Rolly,<sup>6</sup> Plant,<sup>7</sup> and others. However, other investigators found normal or higher figures than for normal control persons.

One of us<sup>8</sup> has studied the specific dynamic action of food in a group of obese patients after a mixed meal and in another group after a protein meal

(see Tables II and III). In the first series the readings were taken at the end of forty-minutes and one and one-half hours after the mixed meal. Subsequently the method was altered and the reading was taken at the end of the first and second hours after ingestion of a protein meal consisting of 100 gm. of broiled chopped beef and 100 c.c. of water. The results of these observations are presented in Figs. 1 and 2. After the mixed meal only in one patient was there observed a complete absence of specific dynamic action of food. This patient was suffering from dystrophia adiposogenitalis. In another patient with a thyroovarian obesity the specific dynamic action of food was considerably increased over the normal. It is rather interesting to note that this patient was intolerant to thyroid therapy. One-half grain of desiccated thyroid gland three times a day for ten days produced marked symptoms of thyrotoxicosis. The stimulating effect of a protein meal was

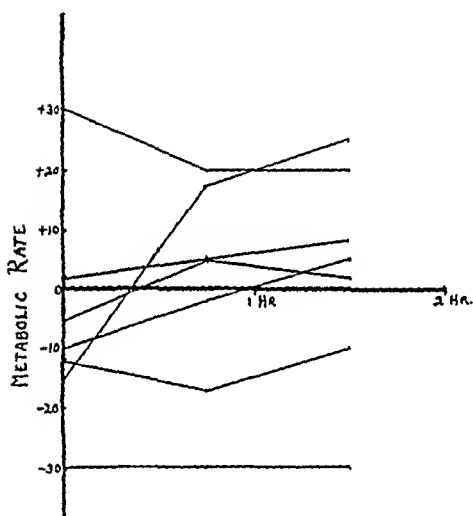


Fig. 1.

Fig. 1.—Effect of ingestion of a mixed meal on heat production.

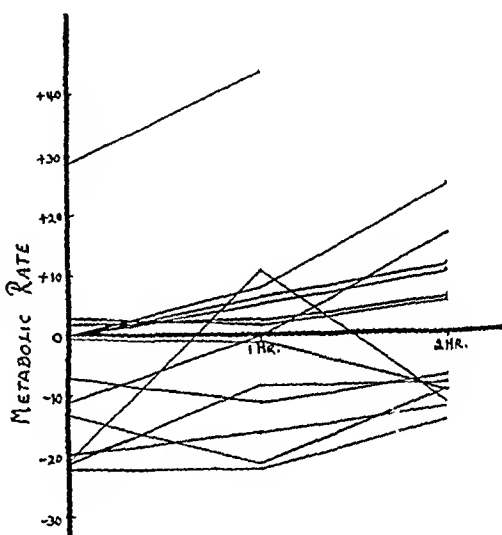


Fig. 2.

Fig. 2.—Effect of ingestion of a protein meal on heat production.

computed in fourteen obese patients; the results were essentially the same as obtains in normal persons with the exception of three patients. Two of these were of the dystrophia adiposogenitalis type and one of obesity incident to menopause. In these three patients the response to the protein meal was low (see Table III). If one bears in mind the great variations in specific dynamic response of normal individuals, it is apparent that no definite opinion in general can be formed as to the rôle this factor plays in the pathogenesis of obesity.

Bernhardt<sup>9</sup> claims to have found long periods of depressed metabolism (negative phases) in the obese after light work and after a meal. Later investigators failed to confirm these findings.<sup>10</sup>

*Water Retention.*—In planning a therapeutic program for an obese person the problem of water metabolism should be taken into account. How great

a loss of body weight may be due to loss of water may be appreciated from the work of Gamble, Ross, and Tisdall.<sup>11</sup> They computed the water weight lost during fasting in terms of fixed bases and thus were able to determine

TABLE II  
EFFECT OF INGESTION OF A MIXED MEAL ON HEAT PRODUCTION

NAME	AGE YEARS	WEIGHT POUNDS	HEIGHT INCHES	BASAL RATE	METABOLIC RATE		PER CENT DIFFER- ENCE FROM BASAL OF PATIENT	
					45 MIN AFTER MEAL	90 MIN AFTER MEAL	45 MIN AFTER MEAL	90 MIN. AFTER MEAL
J. K.	14	199	60	-10	-10	-10	0	0
R. B.	17	159	64	-9	-2	+5	7 (+)	14 (+)
T. B.	18	290	67	+30	+20	+20	8 (-)	8 (-)
N. S.	38	155	58	-12	-17	-10	6 (-)	3 (+)
C. W.	38	237	70	+2	+5	+8	7 (+)	7 (+)
M. H.	41	204	61	-15	+17	+25	18 (+)	48 (+)
L. G.	42	332	64	-5	+5	+2	10 (+)	7 (+)

TABLE III  
EFFECT OF INGESTION OF A PROTEIN MEAL ON HEAT PRODUCTION

AGE	SEX	WEIGHT POUNDS	HEIGHT INCHES	BASAL RATE	METABOLIC RATE		PER CENT DIFFERENCE FROM BASAL OF PATIENT	
					1 HOUR AFTER MEAL	2 HOURS AFTER MEAL	1 HOUR AFTER MEAL	2 HOURS AFTER MEAL
57	F	276	64	-21	-8	-8	13 (+)	13 (+)
29	F	259	66	0	+7	+12	7 (+)	12 (+)
41	F	202	61	-21	+11	-11	32 (+)	10 (+)
15	M	234	67	-11	0	+17	11 (+)	28 (+)
13	M	151	65	+3	+2	+6	1 (-)	3 (+)
12	F	178	60	-22	-22	-14	0	8 (+)
21	F	227	65	-17	-21	-9	8 (-)	4 (+)
44	F	202	62	0	0	-9	0	9 (-)
45	M	270	71	0	+8	+26	8 (+)	26 (+)
32	F	269	64	0	+7	+11	7 (+)	11 (+)
15	F	157	63	-20	-16	-12	4 (+)	8 (+)
20	F	160	61	-7	-11	-7	4 (-)	0
23	M	166	61	+29	+44		15 (+)	
55	F	165	61	+2	+2	+5	0	3 (+)

the amount of water lost from cellular content and intracellular storage. Results in one of their cases, A. G., an epileptic child who fasted for fifteen days, showed that 62 per cent of the loss of body weight was due to loss of water. The figures follow:

Intracellular water loss	cc
Due to destruction of protoplasm	1,620
Due to reduction of cell volume	470
	2,090
Extracellular water loss	720
Total loss of body water	2,810
Loss of body weight	3,920 gm
Body weight loss due to water	62 per cent

In the study of water metabolism there are to be considered four sources of water to the organism and three sources of water elimination. The factors involved in water balance as given by Newburgh and his associates are:<sup>12</sup>

*Water Exchange:*

AVAILABLE WATER		WATER GIVEN OFF	
	GRAMS		GRAMS
A. Water drunk	-----	E. Water of urine	-----
B. Water of food	-----	F. Water of stool	-----
C. Water of oxidation	-----	G. Insensible water	-----
D. Preformed water	-----		

Clinical studies of water balance by computing only fluids ingested and fluids excreted in urine and stool do not represent completely the true conditions of water exchange. Besides the water a person drinks as such and the water contained in food (meats run over 50 per cent water, vegetables over 75 per cent water, etc.), water is formed from oxidation of organic hydrogen of food. Another source of available water is from oxidation of protoplasmic hydrogen during the breaking down of body material in the process of combustion, yielding the so-called preformed water. The total proportion of water formed by oxidation of food is in the neighborhood of 300 gm. for a person at light activity. The quantity of preformed water in the body has a more or less fixed relation to living tissue. With deposition of new tissue there is likewise a retention of a corresponding amount of water. Preformed water under normal conditions is too negligible in amount to be of clinical importance, but in starvation the amount may reach large proportions. In practice one sees patients who are on a strict diet far below energy liberated, and yet they fail to lose weight; the probable explanation of this failure to lose weight is the abnormal retention by the tissues of preformed water. It appears then that patients on subcaloric diets, although actually ingesting less fluids than on higher diets, derive significant amounts of water from preformed sources.

Water lost from the body is represented by urine, stool, and water evaporated from the skin and lungs—insensible loss. Urine usually is regarded as the major source of water loss from the body. However, it should be borne in mind that the insensible loss of water is continuous and under unusual conditions may exceed the amount lost through urine. Newburgh and his associates<sup>13</sup> estimated the daily insensible loss of water in persons at normal activity to average from 1,000 to 1,500 c.c. They also furnished an accurate account of water exchange in connection with the study of the total metabolism in obesity. They found that departures from predicted losses of weight were always accounted for by storage or loss of water.<sup>14</sup> Van Noorden in 1910<sup>15</sup> has already indicated the importance of water and salt restriction in treatment of obesity.

Oertel<sup>16</sup> showed that the quantity of water that entered the organism affects the accumulation and consumption of fat. It was also observed that when the loss of water was greater than the intake the fat accumulated in the body decreased. Zondek<sup>17</sup> described patients who retain water as of the salt water obesity type. Bauer<sup>18</sup> speaks of them as cases of hydrolipomatosis.

In 1931 one of us<sup>19</sup> called attention to the importance of water storage as a contributing factor in obesity and reported satisfactory results from the

intravenous use of salyrgan in a group of patients who failed to lose weight on a strict submaintenance diet, the stationary level of weight being doubtless due to hidden water retention. Rowntree and Brunsting<sup>20</sup> later reported two cases of obesity characterized by striking retention of water and in whom weight loss was induced by salyrgan.

To determine latent water retention in obesity we have been using the Volhard dilution test and the McClure Aldrich skin test.

*Volhard Dilution Test—Method* The patient is given 1,500 c.c. or 1,000 c.c. of tap water on an empty stomach and the urine is collected at hourly intervals for the next four hours. During this test the patient is permitted to be up and around and to carry on his usual duties. The amount collected for four hours is measured, the specific gravity taken, and routine urinalysis is made on the mixed specimen. Patients who show cardiovascular disturbances or whose urine shows some deviation from the normal are eliminated as unfit for this test. According to Pratt,<sup>21</sup> a normal four hour output by the Volhard test is equal to or greater than the intake. A more recent study by Bartels and Blum<sup>22</sup> seems to indicate that an output slightly below the intake may be regarded as normal, and after ingestion of 1,500 c.c., the average output of their control subjects was 1,334 c.c. Accordingly, we have accepted as abnormal only those results which show a negative balance of more than 200 c.c.

*Results* Thirty-six patients from the Ductless Gland Clinic, Temple University Hospital were studied. They were all more than 10 per cent overweight as compared with accepted normal weights. Of this group seven patients or 19 per cent had a greater output of urine than intake of water. Ten patients or 28 per cent had an output within the range of normality. Nineteen patients or 53 per cent had an output definitely less than the intake of fluid, averaging a negative balance of 515 c.c. in four hours. These figures, as given in Table IV, compare favorably with a previous study on dilution tests in obesity published by one of us<sup>23</sup> in 1932. This study preceded that of Mahmud<sup>27</sup> to whom Bartels and Blum in their review of the subject refer as the first to describe studies of this nature.

It would appear that more than half of the obese patients in this study showed a disturbance in the water balance as evidenced by the Volhard dilution test. In the thirty-two subjects more than 10 per cent overweight studied by Bartels and Blum, severe water retention (output less than 1,000 c.c. after 1,500 c.c. intake) occurred in 28 per cent, the number of their patients who had a negative balance of 200 c.c. or more was twenty, or 62 per cent of the group. This latter figure comes closer more closely with our own, and in our opinion represents a truer approximation of the incidence of water retention in obesity. Analysis of our data, as well as that of Bartels and Blum (see Tables V and VI) leads us to disagree with their conclusion that 'obese subjects do not retain fluids to a great degree, as in only 7 per cent was the output less than 1,000 c.c.' Careful study of their data shows that the 7 per cent applies to patients from 0 to 10 per cent overweight and not to patients more than 10 per cent overweight into which class the obese individuals would fall. In comparing Bartels' and Blum's cases with our



owu, it is to be noted that only five of their group were thirty or more pounds overweight, while sixteen patients of our group with heights from 60 to 65 inches were well over 200 pounds in weight. We must conclude, therefore, that our patients as a group represent pathologic obesity more so than do those of Bartels and Blum.

TABLE IV  
VOLHARD DILUTION TESTS IN OBESE PATIENTS

NAME	SEX	AGE	WEIGHT POUNDS	HEIGHT INCHES	INTAKE G.C.	OUTPUT G.C.
M. S.	F	29	248	60	1,500	900
M. Z.	F	50	201	60	1,500	1,400
M. W.	F	48	276	64	1,500	750
M. Z.	F	13	196	63	1,250	1,100
L. R.	M	46	233	74	1,500	1,000
A. B.	F	40	275	62	1,000	1,000
T. B.	M	38	295	67	1,500	850
M. M.	F	42	202	64	1,000	625
E. L.	F	10	96	53½	750*	875
E. L.	F	39	202	63½	1,000	700
G. T.	F	63	184	64	1,500	1,045
L. C.	F	49	179	61	1,500	1,684
N. M.	F	50	192	62	1,500	687
E. K.	F	21	344	63½	1,500	890
M. S.	F	15	178	69	1,000	1,000
L. G.	F	26	210½	64	1,500	1,290
C. W.	F	63	168½	61½	1,000	1,000
H. A.	F	64	180½	59½	1,000	720
L. B.	F	35	210	66½	1,000	500
D. C.	F	32	270	64½	1,500	1,700
G. H.	F	33	170	66	1,000	500
M. D.	F	26	148	61½	1,000	1,200
R. Z.	F	23	140	59	1,000	1,000
M. J.	F	37	183	63½	1,000	1,250
J. R.	F	47	172	63	1,500	1,200
I. G.	M	42	332	62½	1,500	900
N. S.	F	38	155	67	1,500	1,700
N. T.	M	53	190	61	1,500	1,100
M. G.	F	23	200	63	1,500	900
M. H.	F	41	204	64	1,500	1,400
A. B.	F	43	244	64	1,500	1,500
A. H.	F	43	242	63½	1,500	1,350
J. W.	F	22	221	64	1,500	1,521
A. R.	F	42	156	62	1,500	500
B. M.	F	25	179	66	1,000	750
M. G.	F	30	164	63½	1,000	685

\*Could not drink full amount.

*McClure-Aldrich Test.*—*Method:* Two-tenths cubic centimeters of 0.85 per cent aqueous solution of sodium chloride is injected intraeutaneously into the flexor surface of the forearm, inner surface of the thigh, the gluteal region, and the abdomen. A duplicate injection is made in areas that are comparatively free from fat deposits. The end-point of disappearance of the raised wheal is determined by palpation. When the elevation persists for sixty minutes, it is indicative that the tissues do not show increased avidity for water.

*Results:* In 15 patients of our series, both the McClure-Aldrich and the Volhard dilution tests were made. In 6 patients or 40 per cent of the group,

the McClure Aldrich test showed rapid absorption in all areas tested, with definite retention by the Volhard test. In 6 patients, or 40 per cent, the results of the Volhard test were normal, in 3 of these the McClure-Aldrich test showed rapid absorption in all areas tested, and in the remaining 3 the McClure Aldrich test yielded practically normal results. In 3 patients the Volhard test was normal, and the McClure Aldrich test was positive only in one or two places tested (see Table VII)

TABLE V  
(AFTER BARTELS AND BLUM)

RESULTS OF DILUTION TESTS ON THIRTY TWO SUBJECTS WHO WERE MORE THAN 10 PER CENT OVERWEIGHT

OVER WEIGHT POUNDS	CASE NO	FIRST HOUR OUTPUT CC	SECOND HOUR OUTPUT CC	THIRD HOUR OUTPUT CC	FOURTH HOUR OUTPUT CC	TOTAL OUTPUT CC
10	9	275	120	70	10	505
10	35	155	165	135	75	530
10	72	485	205	120	80	900
10	100	300	650	50	100	1,300
11	22	305	350	175	15	1,275
11	24	200	570	90	20	850
12	64	670	620	144	50	1,440
12	70	810	700	450	50	2,000
12	108	1,000	550	150	25	2,025
13	44	420	480	180	55	1,135
13	92	800	825	150	20	1,795
14	103	500	600	300	50	1,450
15	20	315	395	125	30	865
15	105	900	700	150	100	1,850
16	79	285	465	260	95	1,105
16	87	600	600	500	120	1,820
17	96	500	500	500	50	1,550
18	81	900	650	200	150	2,000
20	18	555	670	265	40	1,520
20	111	95	220	235	45	695
		75	200	175	250	1,000
		55	500	575	225	1,115
		195	670	250	70	1,215
21	107	550	650	400	110	1,710
22	3	350	575	210	52	1,187
22	11	165	500	145	25	845
22	60	155	375	70	50	650
24	14	550	260	175	60	1,145
29	24	250	435	490	120	1,295
29	58	350	570	290	110	1,320
31	112	295	380	455	320	1,550
		180	375	150	200	1,565
		270	470	470	225	1,415
38	109	210	580	460	35	1,285
		100	590	435	175	1,300
		170	725	420	75	1,400
		210	605	480	220	1,515
40	110	25	620	185	40	1,080
		175	580	255	90	1,200
		250	655	240	55	1,200
46	112	240	210	122	220	1,200
		265	110	300	200	1,275
		225	295	245	205	1,170
		270	230	250	210	1,070
75	15	170	240	140	115	705
		265	265	450	230	1,310

TABLE VI  
(AFTER BARTELS AND BLUM)

PER CENT OF SUBJECTS IN EACH GROUP WITH OUTPUT OF MORE THAN 1,500 C.C. AND LESS THAN 1,000 C.C. OF URINE

MORE THAN 1,500 C.C.	PER CENT
0 to 10 per cent underweight	33
0 to 10 per cent overweight	40
More than 10 per cent underweight	28
More than 10 per cent overweight	34
LESS THAN 1,000 C.C.	
0 to 10 per cent underweight	23
0 to 10 per cent overweight	7
More than 10 per cent underweight	28
More than 10 per cent overweight	28

TABLE VII  
McCLURE-ALDRICH'S INTRADERMAL SALT SOLUTION TEST IN OBESITY

NAME	SEX	AGE	WEIGHT POUNDS	HEIGHT INCHES	ARM M.	ABDOMEN M.	THIGH M.	GLUTEAL M.	RESULTS OF VOLHARD TEST C.C.
M. S.	F	29	248	60	45	15	50	20	-600
M. Z.	F	50	200	60	55	20	45	50	-100
M. W.	F	48	276	64	--	15	25	18	-750
M. Z.	F	13	196	63	50	--	40	--	-150
L. R.	M	46	233	74	40	25	45	40	-500
A. B.	F	40	275	62	7	7	6	20	0
M. M.	F	42	202	64	13	2	3	29	-375
E. L.	F	10	96	53½	25	20	20	--	+100
E. L.	F	39	202	63½	20	19	25	--	-300
G. T.	F	63	184	64	3	8	8	20	-455
L. C.	F	49	179	61	8	57	57	60	+184
N. M.	F	50	192	62	5	23	29	--	-823
T. B.	M	38	291	67	7	--	10	--	-650
E. K.	F	21	344	63½	10	43	52	35	-610
M. S.	F	15	178	69	12	--	6	2	0

Patients who showed a positive McClure-Aldrich test, and normal Volhard test, were carefully studied for cardiac and renal disturbances but no cause could be assigned for the positive McClure-Aldrich test. The apparent discrepancy between the Volhard test, and the McClure-Aldrich test in these cases suggests that over certain fatty areas of the body there is a greater avidity for water than is manifested by the body as a whole. We realize that this series is too small to permit final judgment, but we feel that the McClure-Aldrich test cannot be relied upon to detect water retention in obesity for clinical purposes with the same degree of accuracy as the Volhard dilution test.

#### CONCLUSIONS

In obesity the law of conservation of energy is operative, i.e., the balance between energy output and energy intake is disturbed. From laboratory tests it cannot be deduced with certainty that the underlying factor of this disturbance is conditioned by an endocrine dysfunction. In a large majority

of obese patients, both the basal metabolic rate and the specific dynamic action of food are within normal limits. Clinically, however, many of these patients may present stigmas of single or more often of mixed endocrinopathy. An additional factor which appears to play an important rôle in obesity is a disturbance of water metabolism which may lead to hidden water retention not evidenced by pitting edema. In planning a therapeutic regime this factor should be taken into consideration. The Volhard dilution test may be used as a simple clinical measure to detect obese patients who tend to retain water.

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## EFFECTS OF HYPERPYREXIA ON THE HUMAN BLOOD COUNT, BLOOD CHEMISTRY AND URINE\*

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ARTIFICIAL fever has now become a recognized adjunct in the treatment of many diseases, including syphilis, multiple sclerosis, acute and chronic arthritis, pelvic inflammatory disease, and asthma. The stimulus of popularity has caused the physiologic aspects of hyperthermia to be investigated carefully; however, contradictory findings have been reported, so that work of this kind is still of interest in obtaining knowledge of the body functions during fever.

Haldane and Priestley<sup>1</sup> in 1905 showed that hot baths produced a deficit of CO<sub>2</sub> in the blood by hyperventilation of the lungs. Barbour<sup>2</sup> demonstrated in 1920 that hot baths increased the fluid content of the blood, a fact that was confirmed by others.<sup>3, 4, 5</sup> Henderson, Prince and Haggard<sup>6</sup> had, in 1918, showed that hyperventilation without fever would produce a slight thinning of the hemoglobin corresponding to the degree of apnea, and this fluid balance of the blood has been developed on a chemical basis.

Following the initial dilution the blood then becomes concentrated during continued fever by loss of fluid through the lungs, sweat glands, and kidneys.<sup>7, 8</sup>

Different reports are to be found concerning the blood sugar values after hyperpyrexia. It is reported<sup>7, 9</sup> that increase in body temperature has a stimulating effect on metabolism as shown by a rise in the respiratory quotient and fall in the blood sugar level. However, Lépine<sup>10</sup> found the blood sugar little altered in fever, and where increased he believed it was due to irritating effects of fever toxins on the fourth ventricle. Freund and Marchand<sup>11</sup> declared that an environment sufficient to raise a rabbit's own temperature will also give hyperglycemia. Dennie,<sup>12</sup> using hot baths, has shown an increase in the blood sugar reading.

Uric acid and nonprotein nitrogen increase and the CO<sub>2</sub> capacity decreases.<sup>8, 12</sup> According to Neymann and Osborne<sup>8</sup> the blood chlorides are slightly increased, but Osborne<sup>13</sup> in a later report with Markson states that either no change or a slight decrease takes place, the amount of chloride lost from the body during an average fever treatment being from 18 to 24 gm.

The effect of hyperpyrexia on the blood count has likewise been carefully studied. Bierman<sup>14</sup> observed an initial fall in white cells followed by an increase, due in part at least to stimulation of the hematopoietic system as evidenced by appearance of immature cells. He confirms the finding of

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Hinsie and Carpenter<sup>16</sup> that the maximum increase occurs about the ninth hour, even after the temperature has dropped to normal. The hemoglobin and red count are increased mainly by dehydration, but in part to stimulation of the bone marrow.<sup>16</sup> The lymphocytic series decrease in accordance with the relative increase in granulocytes.<sup>19</sup>

With this rather conflicting literature we have made a study of some of the physiologic changes taking place during routine hyperpyrexia treatment, hoping that the findings would be of use in evaluating this form of therapy. Our report is based on the average findings in seventy-one treatments including ten patients. Most of these were ambulatory and were selected from the out patient department. The cases included chronic arthritis, hypertension, pelvic abscess, central nervous system syphilis, gonorrheal urethritis, and multiple sclerosis.

*Method*—Production of fever was accomplished by use of a standard hyperpyrexia cabinet, using resistance coils to furnish heat and employing no fans or circulating devices. It was therefore a production of fever by contact of heated air with the body surface. Urine was voided and blood obtained from the antecubital vein for blood count and blood chemistry before entering the cabinet and again after the patient's temperature had returned to normal. Water was given freely but no drugs or salt allowed. The oral temperature was recorded every ten minutes which interfered in no way with the treatment. A few continuous records of axillary temperature were taken but were not very satisfactory. The average length of time at which the temperature was above normal was 3.7 hours. The average peak temperature was 103°. A trained nurse was in constant attendance and recorded the pulse and respiration with each temperature.

*Results*—The urine in most cases showed a tendency to become alkaline, although this was but roughly estimated with litmus. There was always concentration of urine during treatment and some patients were unable to void any urine even after an interval of four hours from the previous voiding. Very little change in the sediment was observed. Occasionally where albumin appeared before the treatment none was seen in the second specimen and in the same manner hyaline casts showed a tendency to decrease during treatment. No sugar was observed in any of the specimens either before or after the period of fever even though in one patient blood sugar readings as high as 190 mg/100 cc were found following return of normal temperature.

Blood chemistry determinations were limited to sugar, nonprotein nitrogen, creatinine, and chlorides. The average readings are as follows:

	BEFORE TREATMENT	AFTER TREATMENT
Sugar	99.9 mg/100 cc	121.3 mg/100 cc
Nonprotein nitrogen	27.4 mg/100 cc	30.8 mg/100 cc
Creatinine	1.27 mg/100 cc	1.4 mg/100 cc
Chlorides	485.0 mg/100 cc	474.1 mg/100 cc

The blood counts included hemoglobin, red cell count, white cell count, polymorphs (filamented and nonfilamented), and lymphocytes. Other types of white cells were not included in computing the averages because of their

infrequent appearance. No reticuloocyte counts were attempted. The average results of the blood counts were as follows:

	BEFORE TREATMENT	AFTER TREATMENT
Red cells	4,530,000/c.mm.	5,258,000/c.mm.
Hemoglobin	85.0%	92.4%
White cells	6,270/c.mm.	10,590/c.mm.
Neutrophile polymorphs	68.1%	81.6%
Filamented	50.2%	58.7%
Nonfilamented	17.9%	22.9%
Lymphocytes	31.9%	18.4%

*Discussion.*—Concentration of the blood during protracted fever may be expected, although Tenney<sup>16</sup> says that if fluids are given abundantly, the viscosity of the blood remains the same. The hemoglobin increased approximately 9 per cent, which we assume to be entirely due to concentration. Reticuloocyte counts would be of value in determining whether stimulation of the red blood forming mechanism actually takes place. Creatinine increased 14 per cent and nonprotein nitrogen 13 per cent, a fact which may indicate some destruction of the body tissue, since, other factors being equal, increase due to concentration alone should approximate the findings of the hemoglobin. Perhaps the most striking finding is the rise in blood sugar. With the known increase in the respiratory quotient during fever, it might be assumed that the blood sugar level would be decreased. Actually we found the average increase to be over 20 per cent. Dennie,<sup>12</sup> who reports the same results, believes this is due to central stimulation through the autonomic system. This phase needs more investigation, especially where stimulation of the adrenals may be the responsible factors. This elevation of blood sugar is transient, and although we have had readings as high as 190 mg./100 c.c., no sugar spilled over into the urine. We substantiated the findings of Markson and Osborne<sup>13</sup> that the chlorides show a slight decrease. The average drop was 2 per cent.

The white cell count showed an increase of 68 per cent, thus allowing 9 per cent as that due to concentration of blood, we find 59 per cent increase which must be accounted for either by stimulation of the white blood forming organs or by a pouring out of these cells from storage depots within the body. We found the increase in nonfilamented cells to be 30 per cent, while myeloblasts, premyelocytes, and myelocytes were occasionally seen, thus indicating considerable stimulation to formation. The remaining 29 per cent of increase is accounted for by response of the reticuloendothelial system to the stimulation of heat as from infection, by pouring into the blood older forms of banded polymorphs. That the bone marrow responds more quickly than the lymphatic system is seen in the relative decrease of lymphocytes in the count following treatment. Stimulation of the lymphatic system occurs but more slowly than the white cell forming organs.

We believe that different methods may account for the different findings of investigators. Thus some of the work was done on animals and some on men. The fever in some cases was due to disease<sup>3,4</sup> and in others the temperature was elevated by means of hot baths, short wave oscillations, electric heat, etc., permitting various factors to enter in. We feel that one of the

most important points in arriving at any conclusion is that a sufficiently large series of cases be studied to account for individual variation. Thus our averages include ten treatments of one patient who reacted very differently from the others, in that the blood sugar showed a decrease and the white cells were displaced by the lymphocytes. This despite the fact that her pelvic abscess was clinically cured.

#### SUMMARY AND CONCLUSIONS

1 Seventy-one treatments of ten patients by artificial fever have been studied for some of the commoner changes in the blood count, urine, and blood chemistry.

2 During treatment, the blood is first diluted, but later becomes concentrated.

3 The urine shows an increasing alkalinity and occasionally clearing of albuminuria.

4 The blood sugar level shows a marked rise, with no glycosuria when the level reaches to the accepted renal threshold or beyond.

5 Creatinine and nonprotein nitrogen show more of an increase than can be considered due to concentration.

6 Blood chlorides decrease approximately 2 per cent.

7 The white blood count is increased by fever treatments. This is first noticed in the granulocytes, and is due partly to production of immature forms and partly to mobilization of older types.

8 Any experimental work of this nature should be under standard conditions and should cover a sufficiently large number of cases to allow for individual variation.

We wish to express our thanks to Miss Esther Rodewald, Miss Muriel Smith, and Miss Dorothy Dixon, who performed the laboratory work, and Miss Blanche Marvin and Miss Ursula Brunner of the physiotherapy department for their careful supervision of the patients while under treatment.

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## THE BLOOD SUGAR IN UNCOMPLICATED AND UNTREATED NEUROSYPHILIS\*

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**S**YPHILIS when present in the human body can act as an irritant, can cripple, or destroy. When it attacks the central nervous system, it usually produces some form of neurosyphilis. In the floor of the third ventricle of the central nervous system there is an area which, when destroyed or irritated, will produce a hyperglycemia. Below it and attached to it is the pituitary gland. Irritative and destructive lesions of certain portions of this gland, especially the posterior and infundibular parts, will produce a hyperglycemia. A lesion of the pituitary may produce hyperglycemia indirectly by affecting the area in the floor of the third ventricle and/or the sympathetic-parasympathetic nervous conduction (and/or connection), or it may act through hormone influence on the thyroid, the suprarenals, or the pancreas.

There have been described lesions due to syphilis and which resulted in hyperglycemia in all of these areas. Hyperglycemia has also been described in abnormal emotional states. In neurosyphilis there is usually an associated abnormal emotional state. In view of these possibilities it was felt that a study of the fasting blood sugar in uncomplicated and untreated neurosyphilis was justified in as much as it might reveal some evidence of an abnormal sugar metabolism.

### RESULTS

The study was composed of the fasting blood sugar of 207 cases of uncomplicated and untreated neurosyphilis. These cases subdivided themselves into 177 cases of general paresis, 22 cases of cerebrospinal syphilis with psychosis, and 12 cases of tabes without psychosis.

The method of estimation of the blood sugar was the Folin-Wu.<sup>1</sup>

Table I shows the salient facts concerning the blood sugar. The figures represent the number of milligrams of sugar in 100 c.c. of whole blood. Normal blood sugar is considered to be within the range of 80 to 120 mg.

\*From the Psychiatric Clinic, Boston State Hospital.  
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TABLE I

TYPE	NO CASES	RANGE VALUES	MEAN	CASES WITHIN NORMAL	PEP CENT	CASES BELOW NORMAL	PEP CENT	CASES ABOVE NORMAL	PEP CENT
Neurosyphilis	207	66 160	95.6	187	90.3	9	4.3	11	5.2
General paresis	177	66 154	97.8	160	90.2	8	4.5	9	5.0
Cerebrospinal syphilis with psychosis	22	66 104	92.9	20	91.8	2	9.1	0	0.0
Tabs without psychosis	12	76 122	100.8	9	74.9	1	8.3	2	16.6

## DISCUSSION

The mean blood sugar values in syphilis of the central nervous system were all within normal limits for the types studied. It sometimes happens that although the mean in a group of estimations is within normal limits so many of the estimations are not within this range that the mean value is worthless and misleading. In the groups studied, however, this was not so. In the group neurosyphilis as a whole 90.3 per cent, in general paresis 90.2 per cent, cerebrospinal syphilis with psychosis 91.8 per cent, and in tabs without psychosis 74.9 per cent of the cases had fasting blood sugar values within normal limits. In those cases which had values below or above normal no pathologic reason for the abnormal fasting sugar could be discovered. It is very possible that these abnormal sugar estimations which constitute less than 10 per cent of the total group are due to some masked pathology of the regions suggested earlier, but lack of definite evidence is not conducive to definite statements in this respect. It is significant, however, that in over 90 per cent of the cases of uncomplicated and untreated neurosyphilis studied there was no evidence of any abnormality of sugar metabolism which could be demonstrated by a fasting blood sugar.

From this study there are two logical conclusions: (a) fasting blood sugar in neurosyphilis is usually within normal limits and (b) when an individual has neurosyphilis it is highly improbable that there will be a coincident abnormality in sugar metabolism which can be established by a fasting blood sugar.

## SUMMARY

There has been presented a study of the fasting blood sugar in 207 cases of neurosyphilis. In 90.3 per cent of these cases this sugar was within normal limits.

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# LABORATORY METHODS

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## A MOUSE PROTECTION TEST FOR STANDARDIZING ANTIMENINGOCOCCUS SERUMS\*

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ABOUT six months ago we reported successful results in using a mouse protection test for standardizing antimeningococcus therapeutic serums.<sup>1</sup> The present paper is a further study of this test with especial reference to its practical application, and a comparison of the results obtained by the mouse protection test and by titrating the serums for agglutinins and precipitins.

The standardization of antimeningococcus therapeutic serums has been the subject of study for quite some time. Until recently the method generally employed was to determine the agglutinin, precipitin and, in some instances, the complement-fixing titer of the serums and consider them potent when they had a high titer of one or more of the above antibacterial antibodies.

The question of the rôle of agglutinins, precipitins, and complement-fixing antibodies in preventing or overcoming infection is still a matter of dispute. It is a well-known fact that in some antibacterial serums, such as antipneumococcus serums, the protective power of the serum does not run parallel with their antibacterial antibody content. The same may also be true of antimeningococcus serums. If a reliable test could be found by means of which the protective power of the serums against virulent cultures of meningococci could be determined, their therapeutic value could probably be more accurately standardized than by antibacterial content determination.

The main difficulty in carrying out protection tests against meningococci is that small laboratory animals are ordinarily not susceptible to meningococci unless large doses are given, and it is difficult to obtain a constant killing dose even with large doses. Zrodowski and Voronina<sup>2</sup> were able to infect rabbits by subarachnoid inoculation of meningococci. In our hands their method gave exceedingly variable results, and we, therefore, found it unsuited for routine tests. About two years ago Miller<sup>3</sup> reported successful infection of mice when recently isolated meningococcus cultures were suspended in a solution of mucin instead of saline and the suspension was injected intraperitoneally. By repeated passage he obtained cultures that killed mice in six to twenty-four hours.

We employed a slight modification of Miller's method to raise the virulence of some of our cultures as follows:

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Recently isolated cultures were maintained on freshly prepared infusion blood agar pH 7.274 and were transplanted twice a week. Before mice were inoculated the cultures were transplanted two or three times at twenty-four hour intervals on large tubes of freshly prepared blood agar, selecting the tubes that contained considerable water of condensation, as such tubes usually gave a heavy mucoid growth within twenty-four hours. The twenty-four hour growth from these tubes was washed off with a mucin solution that was prepared according to Miller's<sup>3</sup> method\*. The mucin suspension of the culture was filtered through a thin layer of sterile cotton in order to eliminate clumps and was then standardized by opacity using McFarland's standard. It was further diluted with mucin to the desired concentration of organisms and 1 c.c. amounts of the various dilutions injected intraperitoneally into several mice on each dose. When the mice died, cultures were made from the heart's blood and mucoid colonies were selected from the growth for further passage.

We were able in this manner to obtain two virulent cultures of meningococci Types I and III which produced fatal septicemia in mice when given in doses of 50 to 100 million cocci. Most of the mice died within eighteen to twenty-four hours and the cultures were recovered from the heart's blood. Some of the mice lingered for thirty to forty-eight hours, and in these mice the culture was seldom found in the heart's blood. Although the fatal dose of these strains is very much larger than that obtained by Miller, unselected white mice weighing 15 to 21 gm. were killed regularly and the cultures were recovered from the heart's blood in most instances.

Having obtained virulent cultures we then tested several preparations of antimeningococcus therapeutic serums for their power of preventing death in mice when a multiple of the killing dose of one of these cultures was given. The serums were diluted with mucin and the dilutions were made so that the desired dose was contained in 0.5 c.c. The culture was similarly diluted. One cubic centimeter syringes were used for injecting the test material and 0.5 c.c. of serum dilution was drawn up in the syringe followed by 0.5 c.c. of the culture, and this was immediately injected into mice.

It was found that the serums were able to protect the mice, in multiple proportions, while there was 80 per cent to 100 per cent mortality among the control mice that received the same amount of culture without the immune serum, and culture plus normal serum. The mice that received a sufficient amount of the immune serum remained well and were discarded, while those that received an insufficient amount of the immune serum, and the control mice, showed symptoms of illness after three to five hours and most of them died in eighteen to twenty-four hours. A small number lingered for twenty-seven to thirty hours and an occasional few recovered at the end of two days.

Our next problem was to determine the optimum method of administering the serum in testing its potency. Fifty mice were divided into 5 groups of 10 mice each. Group 1 was given the serum eighteen hours before the culture. Group 2 was given the serum and culture simultaneously. Groups 3, 4, and 5

\*In a personal communication at a later date Dr. Miller advised the addition of 1 per cent glucose to the mucin which we have used in our later tests.

were given the serum one, three, and five hours after the culture. The results are given in Table I; the best results were obtained when the serum and culture were given simultaneously, although a considerable amount of protection was obtained three hours after the culture was administered and possibly larger

TABLE I\*

MENINGOCOCCUS MOUSE PROTECTION TESTS. COMPARATIVE RESULTS OBTAINED BY DIFFERENT METHODS OF ADMINISTERING IMMUNE SERUM

IMMUNE SERUM PREP. 42	RESULTS					
	SERUM 0.005 C.C. + CULTURE 250 MIL. COCCI			SERUM 0.001 + CULTURE 250 MIL. COCCI		
ADMINISTERED	MICE INOC.	SURVIVED	PRO- TECTED PER CENT	MICE INOC.	SURVIVED	PRO- TECTED PER CENT
18 hr. before culture	5	2	40	5	0	0
Simultaneously	5	5	100	5	4	80
1 hr. after culture	5	5	100	5	1	20
3 hr. after culture	5	3	60	5	0	0
5 hr. after culture	5	1	20	5	0	0

\*Controls—250 million cocci 5 mice none survived.

amounts of serum may have prevented death even at a much later period. Since the best results were obtained in this test when the serum and culture were given simultaneously, we selected this method for our subsequent tests of the protective power of various preparations of antimeningococcus therapeutic serums which were obtained from various sources including several of our own preparations.

We have tested so far 16 different preparations of antimeningococcus serums, 15 of these serums were tested with the culture 23047 which is a Type III, and 6 serums were tested with culture 24995 which is a Type I. Each serum was titrated for agglutinins and for precipitins as well as for its power to prevent death in mice when they were given 200 million cocci of the culture. Five mice were used for each dose of the serums as well as for the controls, where either culture plus normal serum or culture only was used. Unselected white mice were used in these tests, which varied in weight between 15 and 21 gm. Since mice of different weights show considerable difference in their susceptibility to meningococci we included in each group of 5, mice of the same weight in order to make the results of each test comparable. The results are given in Table II.

The results obtained in this experiment show that the agglutinin, and precipitin titers of the serums tested did not run parallel with their power to protect mice against a multiple of the minimum fatal dose of virulent meningococci. As shown in Table II, serums that had approximately equal agglutinin and precipitin titers differed considerably in their protective power (I No. 4 and 5). Also serums that differed widely in their agglutinin and precipitin titers gave the same amount of protection (I No. 6 to 10). On the other hand, serums that were poor in both agglutinins and precipitins were also poor in protective power (I No. 12 to 15).

The five serums that were tested with both Types I and III cultures differed in three instances in their protective power against the two types. This suggests that in standardizing serums it might be well to test against more than one type of culture unless a major strain could be found that would be representative of the different types of meningococci.

TABLE II

Meningococcus Mouse Protection Tests Serum Titrations for Agglutinins, Precipitins, and for Protective Power Against Virulent Cultures

TI ST CHITURE	POLY SERUM		AGGLUT TITER		PRECIP TITER		MOUSE PROTECTION										MOUSE PROT DOSES PER CC	UNITS* PER CC
							SERUM DOSAGE - PERCENTAGE					MICE SURVIVED						
							0.05 CC 1 ET CENT	0.01 CC PER CENT	0.005 CC PER CENT	0.002 CC PER CENT	0.001 CC 1 ET CENT	0.0005 CC PER CENT						
NO	PREP	TYPE III	TYPE III	POLYV														
23047 Type III Dose 200 million cocci	1	304S	1000	60	120					100			100	80 100-		1300	130	
	2	42	4800	15	15					100			80 100*	80		1250	125	
	3	L520H	1800	60	120								100			1000	100	
	4	024665	2000	80	120						100			0†		1000	100	
	5	L527L	2000	80	160						100					710	71	
	6	163	1000	60	40					100			40 60*			500	50	
	7	194	2000	40	40					100			40 60*			500	50	
	8	169 1C	400	60	180					100			40			500	50	
	9	47	3200	32	70					100			40			500	50	
	10	L518A	1200	60	60						100		10			500	50	
	11	45	0400	16	70					100		20				250	25	
	12	70M	800	10	15				80 100*	20		0		0		100	10	
	13	EL1019	800	20	30				80	60		0				100	10	
	14	201	400	8	20				80	60		20				100	10	
	15	202	400	6	20				50	40		20				100	10	
21007 Type I Dose 200 million cocci	1	L527L	6100	80	160					100		60				550	55	
	2	L518A	5500	60	60					100		60				550	55	
	3	024665	4000	60	120					100		40				500	50	
	4	42	9000	15	15				100			20				250	25	
	5	L520H	1800	60	120					80		20				200	20	
	6	47	2000	16	10			80	0			20				20	2	

\* Fifty million cocci give 80 to 100 per cent mortality

† Repeated tests

\* A unit = ten times the smallest amount of serum that will protect 80 per cent of the mice against a standard amount of virulent culture

† The amount of serum in this test was 0.0005 cc

## SUMMARY

Using a slight modification of Miller's method the virulence for mice of two meningococcus cultures Types I and III was increased so that a minimum fatal dose was obtained, a multiple of which was then used to test the protective power of antimeningococcus serums.

Sixteen different preparations of antimeningococcus therapeutic serums were titrated for their ability to prevent death in mice as well as for their agglutinin and precipitin titers.

It was found that the agglutinin and precipitin titers of the serums did run parallel with their protective power although in most instances the highest protection was obtained with the serums that were high in agglutinins or precipitins. The serums that were low in agglutinins and precipitins were, with the exception of one serum, also low in protective power.

The protective power of the serums differed when tested with Type I and Type III.

## CONCLUSION

From the results obtained in this experiment the indications are that the mouse-protection test can be used to standardize the potency of antimeningococcus therapeutic serums. By means of this test a definite unitage of the protective power of the serums could be established which seems reasonable to assume would be a better index of their therapeutic power than the determination of their antibacterial antibody content.

It is suggested that the unit of the protective power of antimeningococcus serum should be ten times the smallest amount of serum which will protect for forty-eight hours 80 per cent to 100 per cent of a series of 5 to 10 mice against a multiple of fatal doses of virulent meningococcus cultures. By adopting a definite unitage in standardizing different preparations more uniform results could be obtained in different laboratories.

A serum of standard unitage should be used as control when new serums are standardized for protection. Such a serum could perhaps be supplied by the Hygienic Laboratories in Washington, D. C., and would serve as control of the validity of the tests.

Further work is in progress to increase the virulence of other types of meningococci and to test immune serums for cross protection against the different types of meningococci; also to find if possible, a major strain that could be employed in the routine testing of the potency of immune serums.

NOTE: Since the completion of this paper Miller<sup>4</sup> and Rake<sup>5</sup> published the results of similar investigations in which they confirmed the value of the mouse-protection test in standardizing antimeningococcus serums.

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## A RAPID SLIDE TEST FOR THE SEROLOGIC DIAGNOSIS OF TYPHOID AND PARATYPHOID FEVERS\*

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FELIX'S<sup>1</sup> contention that antigens of the proteus typhoid, and paratyphoid group consist of two distinct fractions the stable somatic "O" antigen and the labile, flagellar "H" antigen each giving rise to a corresponding agglutinin aroused new interest in the diagnosis of the enteric fevers. The existence of the H and O antigens as separate entities is questioned by Craigie,<sup>2</sup> but from a purely diagnostic standpoint it is relatively unimportant which theory is accepted since the injection into animals of a motile strain of an organism of the enteric group produces an antiserum in which the two types of agglutinins may be demonstrated. In the typhoid and paratyphoid groups the H agglutinin is type specific whereas the O agglutinin is group specific. According to Felix the O agglutinin is indicative of infection while the H agglutinin shows little or no relation to the actual course of the disease.

The high titers for H agglutinins found in the blood of individuals inoculated with T. A. B. vaccines is too well known to merit further comment, but the height to which the O agglutinin titer may rise is not so clear. Felix<sup>3</sup> and Stuart and Krikorian<sup>4</sup> were unable to demonstrate O agglutinins in the blood after prophylactic immunization. On the other hand Gardner,<sup>5</sup> Smith<sup>6</sup> and Wylie<sup>7</sup> found certain vaccinated individuals with O titers as high as 1:250. Recently Dennis and Berberian<sup>8</sup> demonstrated O agglutinins of relatively high titer in the blood of persons inoculated with T. A. B. vaccine. Furthermore they showed that "there was no correlation between the number of previous vaccinations, the interval of time since the last vaccination and the height of the titer, making the establishment of an arbitrary diagnostic titer impossible. Hence a single qualitative receptor analysis is not capable of differentiating between inoculation agglutinins and those due to infection."

A living motile culture can be used to detect the presence of both H and O agglutinins in a serum, whereas a single formalized or phenolized antigen cannot be used for this purpose. With the latter type of antigens there is an inhibition of O agglutination probably due to the organisms being kept apart

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mechanically by the hardened flagella, as pointed out by Craigie. In the Laboratories of the Connecticut State Department of Health living motile cultures have been used in the Widal test for several years and although the results of the tests are satisfactory there are certain technical disadvantages in this method. The strains used in the test must be checked for smoothness every three weeks; moreover, the antigens must be transplanted and standardized daily for use in the tests. It is apparent that all these technical procedures would be eliminated in a rapid slide test using concentrated H and O antigens.

The rapid slide agglutination test, for *Brucella* infection (contagious abortion in cattle), first demonstrated by Gwatkin,<sup>9</sup> was brought to its present state of reliability and accuracy by Huddleson.<sup>10</sup> Lienhardt and Kitzelmann,<sup>11</sup> Palmer and Baker<sup>12</sup> and Welch and Mickle<sup>13, 14</sup> have emphasized the accuracy and efficiency of this test in diagnostic work. The rapid slide method proposed in this paper for the diagnosis of enteric fever follows in general the Huddleson technic.

#### PREPARATION OF ANTIGEN

*Eberthella Typhosa Flagellate (HO).*—A strain of *E. typhosa* of known antigenic make-up which has been carefully checked for smoothness is grown on agar pH 7.0 to 7.2 in Blake bottles for twenty-four hours at 37° C. The agar surface is then covered with not more than 5 ml. of 12 per cent NaCl containing 0.5 per cent formalin. After one or two hours the organisms may be washed from the agar by gently rocking the flasks. The suspension is then filtered through at least eight layers of cheesecloth and centrifuged at high speed in graduated centrifuge tubes for one to two hours to pack the organisms as completely as possible. The supernatant fluid is decanted and retained for later use, and the amount of packed organisms recorded. Just sufficient supernatant fluid is added to each centrifuge tube to pool the organisms. It was found that 5 to 7 ml. could be safely added to each 1 ml. of packed cells. Too much supernatant fluid added at this point will dilute the antigen to a degree where later titration is impossible without further concentration. The suspension is filtered through eight layers of cheesecloth and this highly concentrated suspension constitutes the antigen which is now ready for titration.

Both *Salmonella paratyphi* and *S. schottmuelleri* slide antigens are prepared in a similar manner. All three flagellate antigens are prepared in a similar manner. All three flagellate antigens (*E. typhosa*, *S. paratyphi*, and *S. schottmuelleri*) contain O as well as H antigen.

*Eberthella Typhosa Somatic (O).*—The same strain used in preparation of the flagellate (HO) antigen is used in making the O antigen and is carried out in a similar manner except that the organisms are grown for forty-eight hours at 37° C. rather than for twenty-four hours and are washed from the surface of the agar with 12 per cent NaCl rather than with the formalized salt solution. After centrifugation the supernatant fluid is discarded and the packed bacterial cells are pooled, using just sufficient 0.85 per cent salt solution for

transfer purposes. This concentrated suspension is placed in a large flask and 15 to 20 volumes of 95 per cent alcohol is added, the flask shaken vigorously for ten minutes and incubated at 37° C for twenty-four hours.

At the end of the incubation period the antigen appears as a flocculent white mass from which considerable of the alcohol may be siphoned and discarded. The remaining alcohol is removed by centrifugation. About 5 to 7 ml of 12 per cent salt is added for each 1 ml of packed bacterial cells and the concentrated suspension is ready for titration.



Huddleson dropper.

wire loop and standardized

#### MATERIALS

*Standardized Dropper Pipette*—The standardized dropper pipette used for the slide test is similar to that described by Huddleson<sup>10</sup> and is prepared by drawing out thick-walled glass tubing of  $\frac{1}{8}$  inch bore and cutting the capillary end at 0.07 diameters (B and S gauge). Such a pipette delivers approximately 0.03 ml per drop. A Kahn pipette may be used although the Huddleson dropper is more convenient.

*Glass Slides*—The glass slides are made from ordinary picture frame glass 7 inches long by 5 inches wide and  $\frac{1}{16}$  inch thick. Twenty-eight one-inch wax rings are placed on the slide, four rows of six rings and one row of four, using a mixture of 70 per cent paraffin (m.p. 48° C) and 30 per cent petrolatum heated to 130° to 140° C. The wire loop used to make these rings is prepared by winding tightly No. 28 gauge wire around a one-inch test tube. The loop formed in this manner is wound (single thread) with No. 12 thread and forced

into a regular platinum loop holder. The loop is dipped into the hot wax petrolatum mixture and then placed on the glass slide. With very little practice a single slide can be prepared in one minute. The first two rows of rings are used for O and H (*E. typhosa*) antigen, the third row for *S. paratyphi*, the fourth row for *S. schottmuelleri*, and the fifth row for controls on each antigen. We found it convenient to prepare 50 slides at one time for our investigational work, although it is probable that for routine diagnostic purposes only a few slides prepared in advance would be necessary. Antigen controls are necessary only on the first slide test made each day. Shortly after slides have been read they should be washed off under hot, running water. This removes both the wax rings and the serum antigen mixture. To clean the slides they are rubbed on both sides with Bon Ami, dried and wiped off with a clean cloth. Just before the wax rings are placed on the slides, it is desirable to go over each slide once with a piece of chamois.

#### ANTIGEN TITRATION

In titrating these antigens the desired dilution to be obtained is one that will show an agglutination on the slide which agrees with a similar dilution previously obtained in the tube test. Because the usual range of dilutions used in most macroscopic tube tests is 1:20, 1:40, 1:80, etc., it seemed advisable to concentrate the slide antigens to the point where agreement would be obtained with these dilutions in a tube test. The tube test used for comparison utilized 0.5 ml. of the serum dilutions and 0.5 ml. of antigen. Negative, partial positive and strongly positive agglutinating serums are used for the titration of all antigens. In each of four test tubes is placed 0.5 ml. of the antigen to be titrated, and 0.1, 0.2, 0.4, and 0.5 ml. of the retained supernatant fluid is added. In titrating O antigen 12 per cent salt is used since this supernatant fluid contains flagellated organisms. The diluted antigen in each tube is then tested with serums of known tube test titer, using one drop (0.03 ml.) of antigen and 0.08, 0.04, 0.02, 0.01, 0.005 and 0.002 ml. respectively of each known serum. These dilutions correspond to dilutions of 1:20, 1:40, 1:80, 1:160, 1:320 and 1:640 in the tube test used in these laboratories. The serum and antigen is mixed with pieces of wooden applicators or toothpicks starting with the 0.002 ml. amount of serum working toward the 0.08 ml. amount. The glass slide is then rocked back and forth (usually fifteen to twenty times) and the agglutination read at once by holding the slide over a desk lamp, so that the light is transmitted through the slide but not directly into the observer's eyes. The clumping of the organisms takes place at once with positive serums and it may be observed to increase as the rocking process is carried on. The amount of clumping is estimated as ++++ (complete), +++ (75 per cent), ++ (50 per cent), + (25 per cent), ± (faint to 25 per cent), and 0 (no clumping). The dilution of antigen that shows no clumping with negative serums. complete clumping with a clear background with strongly positive serums in all amounts of serum used and gives the titer with partial positive serums obtained in the tube test will indicate the proper dilution of the prepared antigen. The titers obtained with the slide test should agree with the titers obtained by tube test within a ± to + result. Supernatant fluid

is then added to the antigen in the amount indicated by titration. If considerable evaporation takes place when centrifuging, 0.85 per cent salt solution or distilled water should be used for the diluent since too great a salt concentration adversely affects the dispersion of the antigen.

After titration, sufficient gentian violet and brilliant green are added to all antigens from 1 per cent aqueous stock solutions so that a final concentration of 1:40,000 is obtained with the former dye and 1:20,000 with the latter. These dyes prevent contamination of the antigens during use and facilitate the reading of tests. Five months after preparation the slide antigens prepared in these laboratories still give consistent results.

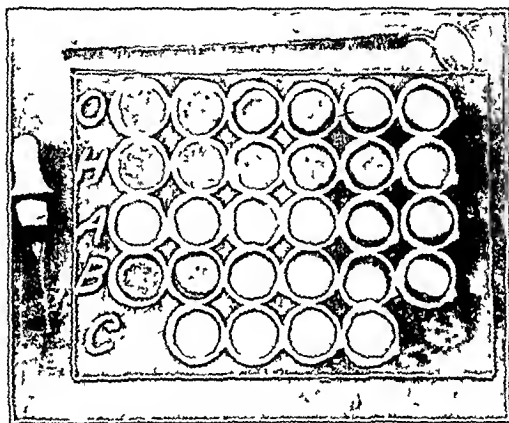


Fig. 2.—Showing typical agglutination with *S. typhosa* O and H antigen negative *S. paratyphi* cross reaction with *S. schottmuelleri* and controls on each antigen.

#### TECHNIC OF TEST

In the routine diagnostic test we have found it more convenient to use all serum amounts described above except the 0.002 ml., i.e., 0.08, 0.04, 0.02, 0.01, and 0.005 ml. of serum giving dilutions corresponding to 1:20, 1:40, 1:80, 1:160, and 1:320 in the tube test. The serum to be tested is pipetted in the above amounts with a Kahn pipette (0.2 ml. graduated in hundredths) into four rows of rings on the glass slide starting with the greatest amount and going from left to right. In the fifth row (used only for the first test each day) 0.08 ml. of 0.85 per cent salt solution is added to each of the four rings. A drop of O antigen is added to each of the serum amounts in the first row and to the first ring in the fifth row (control). Similarly, a drop of H antigen is added to each ring in the second row and the second ring in the fifth row. *S. paratyphi* antigen is added to the third row and *S. schottmuelleri* to the fourth row, each with the appropriate controls in the third and fourth rings respec-

tively in the fifth row. All antigens are shaken gently but well before using. Each row of serum antigen mixture is mixed thoroughly with a separate toothpick or piece of applicator starting with the smallest amount (0.005 ml.) of serum working from right to left.

After mixing, the glass slide is gently rocked back and forth 15 or 20 times. (We usually check the degree of clumping after each five.) The degree of clumping is estimated as noted previously and recorded. The type of clumping obtained with the slide test O antigen does not correspond to the typical small-flaking or granular agglutination obtained in the tube test, and hence O and H agglutination cannot be differentiated by appearance. This is no disadvantage since both types of antigen are used.

#### DISCUSSION

Since the slide test has been developed, 256 serums from patients suspected of having typhoid fever and 200 Wassermann serums have been studied by this method, and the results compared with the macroscopic tube test. In these laboratories a live motile antigen is used in the tube test which is incubated three hours at 56° C. and read after being placed in the refrigerator overnight. Through the courtesy of the New York State Laboratories it was possible to study a further group of 200 Wassermann serums, using the New York *E. typhosa* H and O antigens.

Of the total of 456 serums studied with the slide test and compared with the Connektient method, excellent agreement was obtained. The slide antigens were slightly more specific, in that fewer questionable reactions were obtained in known negative serums. Inasmuch as the Connektient method does not utilize a pure O antigen it seemed advisable to compare the O and H slide antigens with the New York O and H antigens following the New York tube test technique. In a series of 200 Wassermann serums the New York O antigen showed agglutination in 11 per cent, the New York H antigen in 33 per cent, whereas the slide O antigen showed some reaction in 9 per cent and the H in 43 per cent. The degree of agglutination with the New York H antigen was somewhat greater than the slide H antigen. Since the slide antigens were standardized originally against the Connektient tube test antigen, it would appear that these results are in reasonably close agreement.

#### SUMMARY

A rapid slide test is proposed for the routine diagnosis of typhoid and paratyphoid fevers. This test makes use of concentrated antigens and undiluted serum and may be carried out within a few minutes. Comparisons made with standard routine diagnostic tube tests indicate that the rapid method is as accurate and as specific as the tests with which it was compared.

The authors wish to express their appreciation to the Misses Betty Robinton and Sarah B. Whitney for technical assistance.

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## A RAPID METHOD FOR PREPARING ANTIGENS FOR THE WASSERMANN REACTION\*

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THE preparation of an antigen for the Wassermann reaction is generally a time consuming procedure. There have been reported several rapid methods for preparing antigens. Ecker and Sasano (1919) published the results of their method and concluded that suitable antigens could be made by extracting normal heart tissue for one to three hours with boiling alcohol in a reflux condenser. These antigens "had more or less marked fixing power at as high a dilution as 1:200." Kolmer (1928) reported his rapid method for preparing antigen. He extracted 25 gm. of beef heart powder with 200 cc. of ether in an Erlenmeyer flask fitted with a Leibig condenser, boiling two hours. This was followed by extracting the dried powder by boiling for two hours in 95 per cent ethyl alcohol and finally with absolute ethyl alcohol. The method was similar to his original method (1922) except boiling was employed instead of the usual extraction in the incubator for several days. He found that the short method using boiling alcohols may sometimes yield extracts of lesser antigenic sensitiveness, but on the whole were serviceable and satisfactory.

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the conclusion might be drawn that this was a good antigen, but there is one point which is very important and should always be considered, and that is, is the antigen sensitive, and is it specific? To prove that the antigen would meet these qualifications it has been employed in the laboratory for a number of years and approximately 50,000 Wassermann tests run. These Wassermann tests were checked with the Kahn test and the results agree remarkably well. It should be stated, however, that the Wassermann tests using this antigen are considered by this laboratory to be more reliable than the Kahn reaction. Statements of physicians in this state have shown that the Wassermann test as run in this laboratory correlates exceptionally well with the clinical history of the patients.

#### CONCLUSIONS

1. The Soxhlet apparatus is a very efficient and rapid method for extracting the soluble lipoids from beef heart.
2. The time for extracting the lipoids from the beef heart is only eight to ten hours by this method compared with fifteen days by Kolmer's method.
3. Antigens produced by this method are of high antigenic titer and have low anticomplementary and hemolytic units.
4. The antigens have good specificity as determined by comparison with the Kahn precipitation test on at least 50,000 serums.

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## DARK-FIELD ILLUMINATION IN THE DIAGNOSIS OF TUBERCULOSIS AND MALARIA\*

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**D**ARK-FIELD illumination for the study of living organisms has become an accepted practice. Its value in the study of stained films of sputum and blood seems to deserve more attention than it has received. After a little experience with the technic, the search for tubercle bacilli not only takes less time, but there is less likelihood of missing a few scattered organisms, as shown by Schoenheit.<sup>1</sup> The same thing seems to be true of the malaria plasmodium. In both cases the dark-field observation should be confirmed by changing to ordinary



Fig. 1.—Tuberculous sputum with dark-field illumination. The bacilli are conspicuous, but the pus cells are almost invisible. Dark field broadens the image of the bacteria.

transmitted light, using ground glass or smoked glass to reduce the light intensity.

Dark-field examination of tuberculous sputum shows the fuchsin-stained bacilli brightly fluorescent (Fig. 1) while the blue-stained bacteria and pus cells are very inconspicuous. A change to transmitted light shows the red bacilli even with a 4 mm. dry objective, but the oil immersion lens can then be used to verify the details (Fig. 2). In a Giemsa stained blood film the malaria plasmodium is also easily found under dark field, excepting the small ring forms. Here again it is necessary to turn to transmitted light before making a diagnosis. It is, therefore, desirable to be able to change from dark field to light field without much manipulation.

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Most laboratory microscopes can be adapted for dark field with very little expense aside from a powerful light, such as a 6 volt, 108 watt headlight lamp<sup>2</sup> mounted with a condensing lens. Such a light is very satisfactory for alternating current, but for direct current a pointolite lamp, or other powerful source, might be better.

Using a two lens Abbe condensor, N. A. 1.20, and a X40 (4 mm.) objective, N. A. 0.65, it is possible to get dark-field illumination by the use of a center

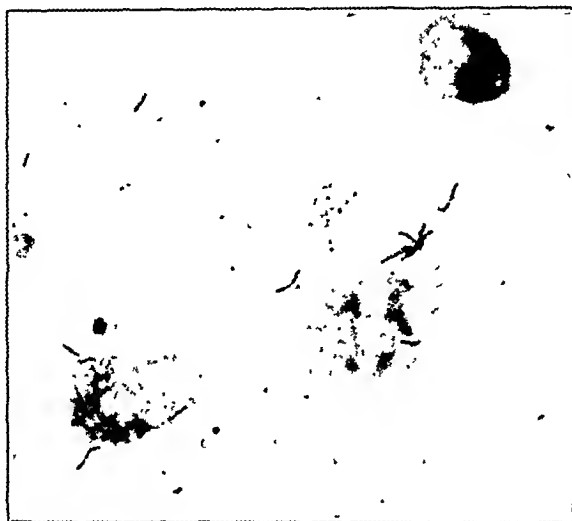


Fig. 2.—Tuberculous sputum with transmitted light. The pus cells are the most conspicuous objects.

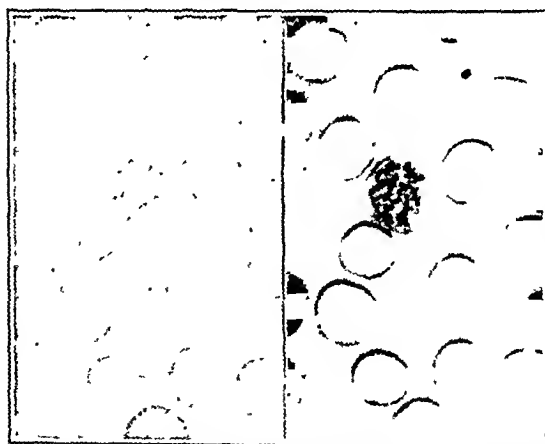


Fig. 3.—Malaria plasmodium in blood cell. Left, as seen with bright field. Right, with dark field.

stop (Fig. 4) below the condensor.<sup>3</sup> If the condensor and slide are connected with a drop of water (oil is too messy), a better dark field may be obtained; but when searching a large film, the dry condensor has the advantage of not getting water into the mechanism of the mechanical stage.

The required size of the center stop depends on the aperture of the objective and on the ocular that is being used. It is well, therefore, to have different

sizes, preferably of metal, but stops for experimental use can be cut out of heavy black paper or thin cardboard. The object slide must be thin enough to permit the substage condensor to focus its outer rays on the film. Some condensers require slides not more than 0.8 mm thick, in any event really thick slides cannot be used.

Dry objectives are very sensitive to incorrect cover glass thickness in dark-field work, so it is better to use only cover glasses between 0.15 and 0.20 mm thick. The cover glass can be mounted on the film with paraffin oil or cedar oil, or with balsam if a permanent preparation is desired.

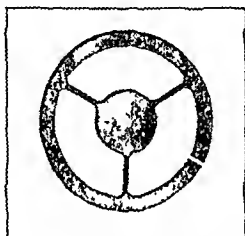


Fig. 4—Center stop for use below condensor to produce dark field. Several sizes should be available such as 12, 16, 18 and 20 mm diameter. The outside diameter of course must fit the microscope in use.



Fig. 5—Visualizing the rays by placing a block of uranium glass on the upper surface of the condensor. The left figure shows the full cone of light with ordinary bright field illumination. The center figure shows a hollow cone of light produced with a small center stop. The right figure shows the effect of a large center stop.

One method of testing the corrections of microscope objectives is by use on a dark field specimen. If therefore the objective gives defective images, from rough usage, dirty lenses, etc., good definition cannot be expected by this method.

As in all dark field work, the cover glass must be clean, for every dust particle is conspicuous under this method of illumination. To clean cover glasses, Stitt's method<sup>4</sup> is good. Immerse the cover glasses in 'Bon Ami' (5 per cent in water) and allow them to dry. Just before use wipe off the Bon Ami. Of course, in using a high power dry lens, a cover glass is essential, but those who have a X20 apochromatic objective should try Coles' method of working without a cover glass, by increasing the length of the microscope tube.

So far it has been assumed that the simplest outfit, only, is at hand. But if the equipment includes a modern oil immersion objective equipped with an iris diaphragm between the lenses, so that the aperture can be reduced to 0.65, it can be used for the dark field, as well as transmitted light, without a cover glass. Of course, the diaphragm should be opened for bright-field study.

Sometimes a green color screen is useful, to remove the secondary spectrum of achromatic objectives. It should be a very transparent green and can be purchased, or made with the dye called "rapid filter green."

This article should not close without mentioning Rheinberg's differential color illumination.<sup>6</sup> By using a dark colored center disc (blue, let us say) and a lighter colored rim (red) the objects on the slide will be colored red while the background is blue.

This method is a beautiful demonstration of the theory of dark-field illumination. Recently, under the name Mikropolychromar, there has been introduced a very accurate, but expensive, attachment for producing this "optical staining."

I take this opportunity to express my appreciation to Craig Howard, of the Department of Bacteriology, University of Cincinnati Medical Department, for placing his many interesting slides at my disposal.

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22 WEST SEVENTH STREET

## BLOOD CALCIUM DETERMINATION, USING STANDARD CALCIUM CHLORIDE SOLUTION\*

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THE normal blood calcium value is very constant from 9 to 11 mg per 100 cc, in children slightly higher, a very close range, yet it is important to know whether in a given patient it is below or over these values, especially in judging borderline cases in which calcium therapy is contemplated.

Five tenths milligrams, certainly as much as 1 mg difference in results over or under that actually present, especially when calcium is found to be below the minimum normal, is of utmost importance to the physician in judging the necessary treatment, particularly in view of therapeutic claims made for certain calcium salts. Erroneous results lead the physician to conclude that the prescribed medication was valueless. In view of the narrow normal threshold, wrong determinations handicap establishment of a diagnosis and line of treatment. A difference of 0.05 cc to 0.1 cc in a deteriorated 0.01 N potassium permanganate solution will show a difference of 0.5 to 1.0 mg of calcium per 100 cc of blood, an error too great.

The most widely employed methods for determination of blood calcium are those of Kramer and Tisdale, and Clark's modification of the former, in both of which 0.01 N potassium permanganate solution is used to titrate in the presence of sulphuric acid the calcium oxalate formed after precipitation with ammonium oxalate. The discrepancies above stated are traceable largely to errors in preparation and standardization of solutions, for too often there is a tendency to assume that the 0.01 N potassium permanganate has remained unchanged since previous determination. We found that volumetric solutions of potassium permanganate had to be checked for each determination, and the ammonium oxalate and oxalic acid used to standardize the permanganate solution also had to be restandardized, although it now is possible to obtain very pure ammonium oxalate and oxalic acid. When it is considered that very small quantities of calcium are dealt with, even the slightest change in titer suffices to offset the result.

As Redwood and Myers indicate, the method of Kramer and Tisdale requires a highly refined technique. In the hands of chemists trained in quantitative procedure and methods of standardization, accurate results are not difficult to obtain. The vexing fact, however, is that application of the methods elaborated by specialists in biologic chemistry often is left to technicians who, though highly conscientious, lack the chemical knowledge of basic principles concerning reactions, standardization and familiarity with quantitative procedure. Owing to insufficient technical training results as high as 15 to 16 mg calcium per 100 cc of blood and as low as 4 to 6 mg in normal cases have been reported in institutions of

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no small standing, despite the fact that the contrary was indicated and later proved to be so. In one hospital laboratory, potassium permanganate solution was purchased and used during an entire month and not restandardized during the interim. The calcium determinations made under such circumstances were so unreliable that, in view of the treatment the patient received, physicians in the pediatric department were at a loss to interpret them.

The methods used for blood calcium determination are somewhat tedious, and in laboratories where only an occasional determination is made, the discrepancy between duplicates may run as high as 1 mg.

In addition to deterioration of 0.01 N potassium permanganate solutions, other factors tending to vitiate results are:

Incomplete washing of the calcium oxalate precipitate, leaving traces of ammonium oxalate, thereby increasing the result.

Incorrect calibration of pipettes used in drawing up the serum; improper correlation between pipettes, burets, and volumetric flasks employed in making 0.01 N potassium permanganate solution from the 0.1 N.

Incomplete centrifugation of the calcium oxalate precipitate and insufficient time allowed for precipitation. Even though one-half hour is given in the method, higher results are obtained if the precipitate is allowed to form in the ice box for several hours or overnight.

Occasionally, unknown to the worker, the wrong anticoagulant is used (potassium or sodium oxalate). Of course, in this case the determination is valueless.

The following modified method is offered to those who have encountered the difficulties above noted, and not to supplant the well-known method of Kramer and Tisdale. Preparation and standardization of 0.01 N potassium permanganate are eliminated without loss of accuracy. The method will find easy application where only occasional calcium determinations are made and facilities are inadequate. It is based upon an easily prepared standard calcium chloride solution which keeps indefinitely. With each blood calcium determination a similar quantity of standard calcium chloride solution is used. The serum and calcium chloride solution are run simultaneously, both being treated alike. The standard calcium chloride solution, containing 0.1 mg. per c.c., serves to give the value of permanganate calcium, from which the serum calcium can be calculated.

*Solutions Required.—1. Standard Calcium Chloride Solution:*

Dissolve 0.2498 gm. of pure calcite (calcium carbonate) Baker's in a little dilute HCl in a wide evaporating dish of 50 to 100 c.c. capacity, care being taken to avoid loss by spattering: (Run the acid down the side of the dish, allowing the reactions to proceed slowly). Carefully evaporate the solution several times to near dryness, each time adding distilled water, and last, evaporate to near dryness, expelling the last traces of HCl. Dissolve the residue in distilled water and dilute to 1 liter; 1 c.c. of this solution equals 0.1 mg. of calcium.

*2. Potassium Permanganate Solution:* Dissolve about 0.030 to 0.040 gm. of potassium permanganate crystals in 100 c.c. of distilled water. Keep in dark bottle. This solution need not be standardized. (A few crystals, the size of

caraway seed, dissolved to a solution sufficiently transparent to read print through, have been found convenient )

### 3 *Three Per Cent Ammonium Oxalate Solution*

4 Approximately N/1 sulphuric acid 28 cc  $H_2SO_4$  added to 970 cc of distilled water

5 *Two Per Cent Ammonia Water* If sodium citrate is used as anticoagulant, correction for the dilution should be made when final calculation is completed (Textbooks omit this statement, suggesting that 1 cc of saturated sodium citrate solution can be used to 10 cc of blood )

*Procedure*—Place in a conical centrifuge tube 0.5 cc of ammonium oxalate solution and 2 cc of citrated plasma Then add another 0.5 cc of ammonium oxalate solution and make up to 6 cc Mix\* thoroughly with distilled water and let stand for one half hour Allowing the precipitate to form in the ice box over night is recommended by other workers, as it yields higher results This we also have found to be the case Centrifugalize at moderate speed (1500-1800 rpm) until sedimentation is complete and carefully pipette off the supernatant fluid, leaving 0.3 cc in the tube After the precipitate is well packed in the tube apex we have found that the supernatant fluid can be decanted and the tube drained in the upright position mouth downward upon filter paper The precipitate is retained undisturbed † Repeat the washing not less than three times, using about 3 to 4 cc of ammonia water, thoroughly stirring the precipitate by rotary motion of the tube Use no stopper! After each washing centrifugation should be continued until the precipitate is firmly packed in the conical tube end Last, dissolve the precipitate in 2 cc of N/1 sulphuric acid by placing in a water bath and heating to 75° C Simultaneously, employ 2 cc of calcium chloride solution and treat exactly the same as the serum

Titrate with potassium permanganate solution made for the purpose It is best to titrate the standard calcium chloride solution first to a faint pink and then match this tint with that obtained in titrating the calcium serum

#### *Calculation*

$$\frac{C \times S}{P} \times 50 = \text{serum calcium}$$

C = mg of calcium in 2 cc of standard calcium chloride solution (0.2 mg)  
 S = cc of  $KMnO_4$  used in titrating the serum  
 P = cc of  $KMnO_4$  used in titrating the standard calcium chloride solution

#### *Example*

$$\begin{aligned} 12 \text{ cc of potassium permanganate solution used for 2 cc of standard calcium chloride solution} &= P \\ 115 \text{ cc of potassium permanganate solution used for 2 cc of serum} &= S \\ 2 \text{ cc of standard calcium chloride solution contains } 0.2 \text{ mg of calcium} &= C \\ 0.2 \times 115 = 12 \times 50 &= 958 \text{ mg calcium per 100 cc of blood} \end{aligned}$$

#### CONCLUSIONS

- 1 Accurate, dependable and easily performed method Steps simplified
- 2 The potassium permanganate solution used need not be standardized It

\*Adding the oxalate solution first and last permits thorough admixture of serum and oxalate otherwise much more time is required uniformly to mix the liquids

†Tisdale used this simplified procedure with a centrifuge tube not over 6 to 7 mm. at the 0.1 mark Centrifuge tubes with wide apices should not be used

can be made just prior to a given determination and used immediately. Other volumetric solutions heretofore employed, dispensed with.

3. The principle of this method is based upon a standard calcium chloride solution, easily made, and keeps indefinitely.

4. Of value where insufficient blood has been drawn for duplicates, as in children.

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### A MECHANICAL DEVICE FOR PREPARING FINE SUSPENSIONS OF TUBERCLE BACILLI AND OTHER MICROORGANISMS\*

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H. J. CORPER, M.D., PH.D., AND MAURICE L. COHN, PH.D., DENVER, COLO.

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THE preparation of fine suspensions of tubercle bacilli is encumbered by many detailed obstacles which retarded accurate quantitative studies with these bacilli for a great many years. In retrospect, one is reminded of the schemes which involved filtration, centrifugation, and numerous other procedures and which left the experimentalist with a feeling of uncertainty as to the actual amount of bacilli present in completed suspensions. Finally, after 1918,<sup>1</sup> reproducible suspensions of tubercle bacilli were prepared by us<sup>2</sup> by slow and laborious manual manipulation, which revealed the presence of about a billion bacilli per milligram moist weight of culture. This method was adhered to until quite recently when it was found that the addition of a few drops of 0.5 per cent solution of sodium taurocholate (because of its surface tension lowering properties<sup>3</sup>) in initiating grinding would materially assist in speeding the operation and give more consistent results for obtaining fine suspensions. With this improvement, obviating the frequent tendency to lumping of the bacilli on the first addition of liquid (0.9 per cent sodium chloride or other watery solutions), the method as now recommended is briefly: about 10 mg. of moist young culture (about three to five weeks old) is accurately weighed in a sterile graduated 15 c.c. centrifuge tube; the bacilli are carefully ground on the glass tube by means of a sterile rounded glass rod slightly smaller than the bowl of the tube; then one drop of sterile 0.5 per cent sodium taurocholate solution is added and the bacilli are thoroughly ground in this with a continual rotary motion; finally, 4 or 5 drops more of the taurocholate solution are added, a drop at a time after each grinding;

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\*From the Research Department, National Jewish Hospital at Denver.  
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after the grinding is completed, requiring usually five to ten minutes, 0.9 per cent sterile salt solution is added first by drops and mixed carefully with the bacilli and finally enough to give the desired concentration of the suspension. The final suspension from which further dilutions are to be made must possess a uniform milky opalescence without lumps or particles and contain a definite amount of bacilli per cubic centimeter. The dilutions are then made with sterile saline solution in stages of one to ten. In order to avoid contamination while manipulating the dilutions, it is well to have a separate small flask of saline solution for each dilution to be prepared. It is also

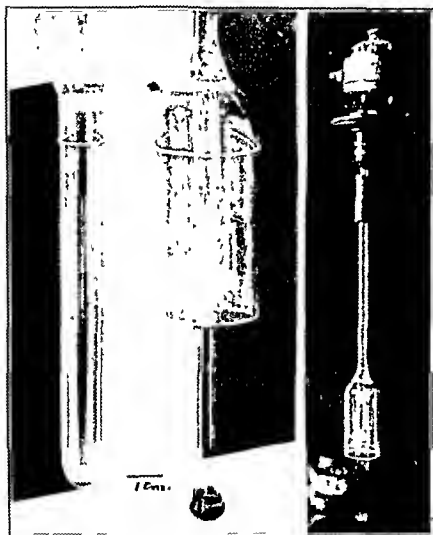


Fig. 1



Fig. 2.

Fig. 1.—Shows the glass parts of the grinding apparatus at the left, and on the right with motor attached during the operation of grinding to make a fine bacillary suspension from a weighed amount of culture.

Fig. 2.—Photograph taken by transmitted light showing the condition of a satisfactory fine suspension of tubercle bacilli with no flocculation at this time, on the left, and an unsatisfactory suspension, on the right, examined six hours after they were prepared from the same culture and containing 1 mg. of bacilli per cubic centimeter. Note the definite flocculation of the bacilli in the unsatisfactory suspension which became evident one hour after standing.

advisable to use individual 1 c.c. sterile pipettes for each transfer, so that 1 c.c. is transferred to a 15 c.c. centrifuge tube, and to this is added the 9 c.c. of sterile saline solution.

It appeared that a further improvement in technique might result from the use of an apparatus devised for this purpose by Dr. Benjamin Sher of the City of Chicago Municipal Tuberculosis Sanitarium, who had not described it but had used it for grinding large amounts of tubercle bacilli. Dr. Sher



kindly submitted the original apparatus for our trial, on which plan the illustrated apparatus used in the tests reported below was built. He also consented to the publication of this description by us so that it might be available to others interested in making uniform suspensions of bacilli. As shown in the illustrations, the apparatus, entirely constructed of Pyrex glass, consists of a long glass tube (about 15 inches long) with pestle end ground carefully into the floor of a Pyrex test tube about six inches long and calibrated to 10 c.c. Above the height of the test tube length is welded a glass hood to prevent contamination. Actually in preparing this apparatus, the hood is inner sealed on the lower portion (pestle) and finally the upper glass tube attached.<sup>1</sup>

In using the grinding apparatus, it is sterilized either by dry heat or autoclaving. It is attached to a slowly revolving laboratory stirring motor by means of a piece of heavy wall rubber tubing. The bacilli to be ground are weighed in the bottom of the test tube, a drop of 0.5 per cent sodium taurocholate solution is added, and the mass is ground for two to five minutes, after which it is diluted as described for the manual procedure. In order to compare the results made by the manual procedure with that made with the motor, a series of suspensions of different cultures at different ages and from different strains of tubercle bacilli (human, bovine, and avian; virulent and avirulent) were compared by means of the growth limit culture test.<sup>2</sup> The results are presented in Table I.

TABLE I

THE EFFICIENCY OF THE MOTOR-DRIVEN GRINDER FOR PREPARING FINE SUSPENSIONS OF TUBERCLE BACILLI

STRAIN OF BACILLI† AND AGE OF CULTURE	CONCENTRATION OF BACILLI IN MILLIGRAMS PER CUBIC CENTIMETER SEEDED ON INSPISSATED EGG YOLK MEDIUM					
	10 <sup>-4</sup> *		10 <sup>-6</sup> *		10 <sup>-8</sup> *	
	MANUAL PREP.	MOTOR PREP.	MANUAL PREP.	MOTOR PREP.	MANUAL PREP.	MOTOR PREP.
Virulent human tubercle bacilli No. 7 (28 days)	3-3‡	3-3	2-3	3-3	1-4	1-5
Avirulent human tubercle bacilli (19 days)	3-3	3-3	3-4	3-3	1-4	2-4
Virulent bovine tubercle bacilli No. 39 (38 days)	3-3	3-3	3-4	3-4	0	0
Avirulent bovine tubercle bacilli BCG (28 days)	3-3	3-3	3-4	3-4	1-6	1-5
Avian tubercle bacilli No. 3 (37 days)	3-3	3-3	3-4	3-4	2-4	3-5

\*The amount of suspension per cubic centimeter is as follows: 10<sup>-4</sup> = 0.0001; 10<sup>-6</sup> = 0.000,001; and 10<sup>-8</sup> = 0.000,000,001.

†The first numeral indicates the number of cultures; the second numeral, the number of weeks incubation appeared.

‡The tabulation of bacilli tested in this series.

\*We are indebted to

The apparatus is the property of the University of Chicago.

ated by the exponents as follows: E.

a total of 3) positive and the first positive culture

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The results recorded in Table I indicate that motor grinding can be employed with equal efficiency to manual grinding for the preparation of fine suspensions of tubercle bacilli. It is also noted, in conformity with previously recorded observations, that bovine tubercle bacilli do not lend themselves quite as well to obtaining fine suspensions as do cultures of human or avian tubercle bacilli or of BCG.

#### SUMMARY

The method of preparing reproducible graded fine suspensions of tubercle bacilli is described. A new apparatus for grinding these bacilli mechanically is presented which gives results equal to those obtained with careful manual grinding and is timesaving.

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### MODIFICATIONS IN THE COLORIMETRIC DETERMINATION OF THE PLASMA PROTEINS BY THE FOLIN PHENOL REAGENT\*

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ANDERSECH and Gibson<sup>1</sup> have suggested certain modifications in the determination of the plasma proteins as a result of their interesting observation that heating the blood proteins for a short time in an alkaline solution intensifies the degree of color obtained with the Folin phenol reagent. The writers, on reinvestigating this subject, reached somewhat different conclusions, and did not obtain the same tyrosine conversion factors as were found by Andersech and Gibson. Therefore, it appears desirable to present briefly the results that were obtained and to describe certain modifications in the determination of total serum protein, albumin, globulin, and fibrin, which were suggested by this work.

That heating considerably increases the chromogenic values which are obtained with the serum proteins was readily confirmed, but a considerably shorter time of heating was found to be sufficient to obtain a maximum color than the half hour recommended by Andersech and Gibson. Representative results on the change in color value obtained on heating human and dog serums are shown in Fig. 1. As this figure shows, a constant color is reached within five minutes on heating the protein in a boiling water bath. However, in developing the procedures of the revised method to allow for a margin of

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\*From the Division of Biochemistry, University of California Medical School.  
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safety, it was decided to employ ten minutes for heating, a time which has proved to be amply satisfactory. The innovation of heating distinctly improves the colorimetric method, in that, more reproducible color values are obtained and samples of serum which are several days old can now be successfully used for analysis without the loss in color which had been noted in the older procedure.<sup>4</sup>

Andersch and Gibson propose to return to the use of  $(\text{NH}_4)_2\text{SO}_4$  for the salting out of the proteins, rather than continue with  $\text{Na}_2\text{SO}_4$  which requires a temperature of over  $30^\circ$  to keep it in solution in the required concentration. In our estimation, this change is not desirable because more time is required, and it is more difficult to filter off the precipitated globulin. With the salt

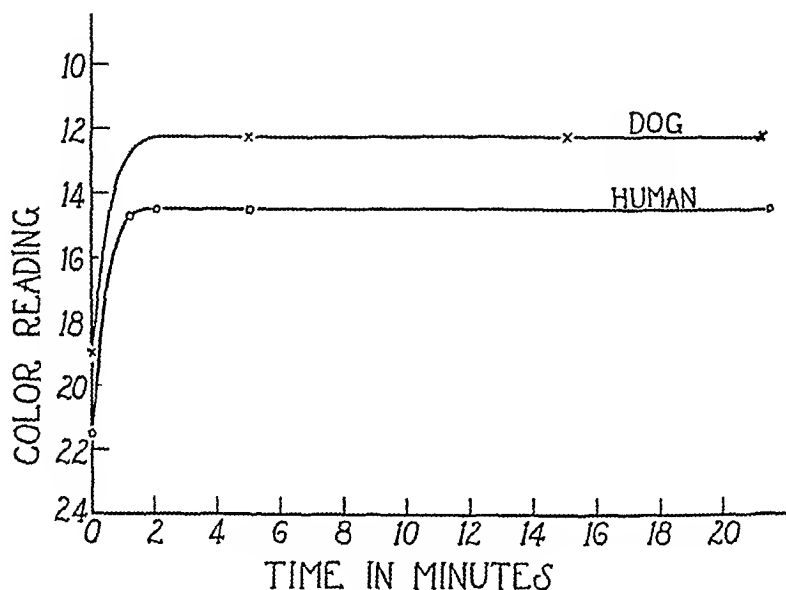


Fig. 1.

suggested by Tuchman and Sobotka,<sup>2</sup>  $\text{Mg Na}_2(\text{SO}_4)_2$ , magnesium oxide is precipitated in alkaline solution, which makes it unsuitable for use with the present colorimetric method.

The previously mentioned authors continue to use the original Folin phenol reagent to secure color development. In our estimation, the improved reagent of Folin and Ciocalteu<sup>3</sup> is to be preferred, as it avoids the development of turbid solutions. A comparison of the tyrosine factors given in this paper with those of Andersch and Gibson indicates also that even with the new heating procedure, a somewhat greater color development is obtained with the Folin-Ciocalteu reagent.

#### EXPERIMENTAL

Except for the heating in alkaline solution, the procedures and the reagents used are, in general, the same as those described by Greenberg,<sup>4</sup> and are published in the laboratory textbooks of Hawk and Bergeim,<sup>5</sup> Peters and Van

Slyke<sup>6</sup> A convenient bath in which to carry out the heating is formed by a large beaker covered with a metal plate containing holes of sufficient size to hold the test tubes suspended in the water without allowing them to slip through. The water should be kept boiling briskly during the heating period.

*Total Protein*—The serum is diluted in the proportion of 1 to 10 with 0.9 per cent NaCl. Pipette 2 cc of the diluted serum into a 20 cc test tube, and add about 5 cc of H<sub>2</sub>O and 2 cc of 5 N NaOH. Mix the contents and heat in the boiling water bath for ten minutes. Now insert a funnel into a 50 cc graduated flask and transfer the contents of the tube into the flask, washing out the tube with several portions of distilled water. Add 3 cc of the phenol reagent made up to the graduation mark and carry out the color comparison against a similar standard containing 5 cc of the standard tyrosine solution.

*Albumin and Globulin*—The globulin from 0.5 cc of protein is salted out in the manner described by Greenberg. Five cubic centimeters of the filtrate containing the albumin are pipetted into a 20 cc test tube, then 5 cc of water and 2 cc of 5 N NaOH are added. After mixing the contents the tube is heated in the boiling water bath for ten minutes. Dilution with water is desirable because otherwise the high concentration of sodium salt present causes a precipitation of some of the albumin. The rest of the procedure is the same as is given above for the total protein.

To estimate the globulin, after washing the precipitated globulin on the filter paper with Na<sub>2</sub>SO<sub>4</sub> solution insert the funnel into a test tube of 25 to 30 cc capacity. Puncture a small hole in the bottom of the filter paper and wash down all the globulin with approximately 0.01 N NaOH. Then unfold the paper and wash off any adhering protein into the test tube. The total volume resulting from this should not be more than 15 or 20 cc. Now add 2 cc of 5 N NaOH and heat the tube on the boiling water bath for ten minutes. Next, allow the contents of the tube to cool, decant into a 50 cc volumetric flask, and wash out with a few cubic centimeters of water. Add 3 cc of phenol reagent and compute as usual.

*Fibrin*—The plasma from oxalated blood is used and the fibrin is separated after the manner of Cullen and Van Slyke.<sup>7</sup> Pipette 1 cc of plasma into a 50 cc cylinder, and add 30 or 40 cc of 0.9 per cent NaCl and 1 cc of 2.5 per cent CaCl<sub>2</sub> solution (prepared from the anhydrous salt). Mix the contents and allow the solution to stand for twenty to thirty minutes. The clot is picked up by gently rotating a glass rod through the clotted solution. Remove the clot to a dry piece of filter paper and press out the adhering liquor as completely as possible. Place the dry protein into a conical 15 cc centrifuge tube, and add 10 cc of water and 1 cc of 5 N NaOH. Now mix and heat the tube on the boiling water-bath for ten minutes. The fibrin will be dissolved, leaving behind a suspension of calcium oxalate. This is centrifuged down and the supernatant liquid is transferred to a 25 cc volumetric flask. Wash out the tube with water and transfer the wash water to the volumetric flask through a small filter to prevent the transfer of the calcium oxalate. Now add 1.5 cc of phenol reagent and compare against a standard in a 50 cc volumetric flask containing 3 cc of the tyrosine solution.

*Conversion Factors.*—The factors to convert the colorimetric readings to their respective protein values and their standard deviations are given in Table I. The figures are based on the analyses of the blood of eight normal young human adults and five dogs. The factors were determined by running parallel nitrogen determinations on the blood proteins by the micro-Kjeldahl method of Pregl. In these determinations total protein and albumin were determined by precipitating the protein fractions with 10 per cent trichloroacetic acid previous to digestion and washing out the nonprotein nitrogen. Globulin was estimated by difference.

TABLE I

TYROSINE EQUIVALENTS FOR CONVERTING COLORIMETRIC READINGS TO GRAMS OF RESPECTIVE PERCENTAGE OF PROTEIN<sup>\*</sup>

		TOTAL PROTEIN	ALBUMIN	GLOBULIN	FIBRIN
Human	F	11.5 ± 0.2	10.5 ± 0.2	11.5 ± 0.25	11.55 ± 0.2
	A	5.75	4.72	2.10	0.347
Dog	F	11.35 ± 0.25	11.6 ± 0.4	10.05 ± 0.4	11.1 ± 0.45
	A	5.68	4.64	2.01	0.333

<sup>\*</sup>Doubt has been thrown on the validity of the tyrosine method of estimating the serum proteins in the blood of patients suffering from nephritis and nephrosis by Tuchman and Sobotka because it is claimed there is an alteration in the values of the tyrosine equivalents. We have not had the opportunity to test this point with our revised procedures. However, Andersch and Gibson report satisfactory results on such patients when the proteins are heated in alkaline solution.

The factors under the heading F are for use in the equation,

$$\frac{S}{U} \times T \times \frac{100}{V} \times \frac{F}{1,000} = \text{protein (in per cent)},$$

in which S is the setting of the standard, U is the reading of the unknown, T is the mg. of tyrosine in the standard solution, V is the aliquot volume of serum or of plasma used, and F is the factor for the particular protein fraction which is being analyzed. If the values of T and V are maintained at the levels described in the procedures given above, namely, T at the value of 1 mg. of tyrosine for the analysis of total protein, albumin, and globulin, and 0.6 mg. for fibrin, and the sample values of V are kept at 0.2 c.c. for total protein, 0.25 c.c. for albumin, 0.5 c.c. for globulin, and 1 c.c. for fibrin, then the equation may be reduced to the form

$$\frac{S}{U} \times A = \text{protein (in per cent)}.$$

The values of A for the respective protein fractions are given under this heading in Table I.

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## A SIMPLE, EFFICIENT, AND INEXPENSIVE DEVICE FOR DRYING PIPETTES AND OTHER LABORATORY GLASSWARE\*

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HENRY FOY, SALONIKA, GREECE

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A SIMPLE and easily manipulated pipette drier can be made from the ordinary electric hair drier, as used by ladies' hair dressers for drying hair. This instrument produces a rapid stream of hot or cold air which it blows out



Fig. 1.

with considerable force, and pipettes can be dried by means of this instrument in a few seconds, six or eight at a time. All that is necessary is to fit a tin

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\*From the League of Nations Malaria Research Laboratory.  
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nozzle over the spout of the blower, and to fit a cork into this nozzle in which six or eight holes are bored to take pipettes of various sizes. We have used an ordinary piece of pliable zinc, about 4 or 6 inches long, turned into a tube by rolling, and bound round the exit of the blower with copper wire; no doubt a more elegant arrangement could be devised, but this is quite satisfactory. We have placed our blower on a wooden base, with the blowing end uppermost, as shown in Fig. 1; if such a device is not used the machine is awkward to hold still on account of its shape.

It is as well not to let the hot air run for more than a few seconds, as the nozzle of the blower being closed, the machine is likely to get very hot, and ruin the working parts, besides the danger of breaking the pipettes. A few seconds of hot air followed by a few of cold are sufficient to dry acetoned pipettes. By means of this device hundreds of pipettes can be dried in a very short time. We have used one of these in this laboratory for the past three years without any trouble whatever.

# DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

**MENINGITIS** Meningococcal and Non Meningococcal in New Born and in Young Infants,  
Ravid J M. *Am J Dis Child* 49 1282, 1935

Meningitis in the newborn and in young infants is characterized by being atypical, protean, and masked in its clinical manifestations.

The diagnosis is often missed and the condition mistaken for acute gastroenteritis, congenital debility, marasmus, tetany, etc.

Illustrative cases are reported in which signs of involvement of the central nervous system were scant or absent and in which the clinical picture centered around the gastrointestinal or respiratory systems.

A review of the literature on this subject was made which emphasized the relative rarity of meningitis at this age. A statistical study of all cases of meningitis in children during a four year period at the New York Post Graduate Medical School and Hospital showed that only 2.5 per cent of the cases of the meningococcal and 4.1 per cent of those of the nonmeningococcal variety occurred in infants three months of age and younger.

The clinical picture as it differs from that in older children and in adults is given in detail. In the absence of pathognomonic signs and when meningeal signs are not found, the following nonspecific signs and symptoms may be suggestive of meningitis: an irregular fever in the presence of gastrointestinal disturbances of any sort which occur without any demonstrable cause and which do not respond readily to treatment, in addition, hyperesthesia, irritability, somnolence, tenseness or bulging of the fontanel, distention of the veins of the neck, incessant crying and a dissociation between the pulse and respiratory rates and the temperature.

The pathogenesis of meningococcal and nonmeningococcal meningitis is discussed. The various portals and modes of entry and sources and means of spread of infection are cited. The different causative organisms of pyogenic meningitis and their relative frequency are reviewed.

The prevalence of meningitis due to *B. coli* and related organisms in the newborn and its rarity in older children is emphasized, and explanatory factors are cited.

The anatomicopathologic features are practically the same in the newborn as in older children.

The prognosis of all forms of meningitis, especially of the pyogenic varieties at this age, is extremely poor. Mortality figures for each variety are given, the lowest being for meningococcal meningitis which is 48 per cent or higher. The high mortality from meningococcal meningitis is due mainly to the difficulty of diagnosis and the delay in the institution of treatment. Cases of recovery from nonmeningococcal meningitis that was spontaneous or that followed specific or nonspecific treatment are cited from the literature.

A diagnosis in the absence of any pathognomonic signs is extremely difficult. Some of the signs and symptoms that may be helpful are cited. The most reliable diagnostic method is still spinal puncture, and it should be resorted to frequently.

The treatment of meningococcal meningitis in the newborn and in young infants is the same as in older children, the conservative line of treatment being the safest and best one to follow. The ventricular and external routes should not be used indiscriminately and should be resorted to only in event of blockage of the circulation of the cerebrospinal fluid. Treatment of nonmeningococcal meningitis is less encouraging but there are nevertheless some indications that new methods of treatment forecast a more favorable progress in this variety also.



**HYPERGLYCEMIA**, Evaluation of, in the Treatment of Diabetes, Mosenthal, H. O. J. A. M. A. 105: 484, 1935.

High blood sugar in the absence of glycosuria is not due to diminished excretory activity of the kidney, nor is it significant of nephritis, but it is a harmless anomaly resulting from an unusually active reabsorption of sugar from the urine in the renal tubules.

While a normal blood sugar level and freedom from glycosuria are among the ideal objectives of therapy in diabetes, there are certain circumstances in which hyperglycemia and glycosuria should not be completely corrected:

a. A normal blood sugar level and freedom from glycosuria should not be gained by underfeeding, since malnutrition favors the development of arteriosclerosis and is one of the contributory factors in producing diabetic coma.

b. Small amounts of sugar may be eliminated in the urine without detrimental results, as shown in renal glycosuria. It is important to recognize this because, in cases of diabetes with a comparatively low renal threshold, there is with rigorous insulin control the possibility of repeated hypoglycemic reactions, which in all probability are injurious in both their immediate and their remote effects and, consequently, should be avoided even at the sacrifice of intermittent glycosuria.

c. Hyperglycemia without glycosuria, according to the available evidence, not only has no damaging effect on the heart and other tissues but is a necessary stimulus for the proper assimilation and oxidation of dextrose in many persons, both diabetic and nondiabetic.

A prolonged, marked glycosuria with its attendant polyuria and dehydration is responsible for the diminished resistance to infectious processes, arteriosclerosis, formation of cataract, malnutrition, acidosis and coma, which are so characteristic of diabetes. Hyperglycemia without glycosuria is not a cause for these complications in diabetes.

**PYURIA**, Significance of, in Children, Hepler, A. B., and Scott, R. T. J. A. M. A. 105: 499, 1935.

There is a tendency among pediatricians to evaluate the importance of pyuria in children on a quantitative basis and to assume that a few pus cells are normal or indicate contamination and that a large number point to urinary tract infection or disease.

Various arbitrary limits of normal are given without qualification as to the method of collection or examination. No systemic study of a large number of children has been reported that would definitely confirm or refute many of the assumptions that appear in the literature on this subject.

To this end 694 infants and children were examined during a period of nine months in the Children's Orthopedic Hospital, from which study we conclude that:

Catheterization is imperative in urinary diagnosis in children. Ninety-nine per cent of 692 children had pus in the voided urine and only 13 per cent in the catheterized.

The amount of pus in a urine properly collected is no indication either of the kind or of the severity of urinary tract disease. In the twenty-four children in this group with demonstrable urinary tract disease, exactly similar lesions existed with pus counts that varied from less than 1 per high dry field to more than 20 per high dry field. Fifty per cent of the children with demonstrable urinary tract disease and a number with advanced lesions and severe infections had pus in amounts well under what is frequently set forth as a normal count.

If a persistent or recurrent pyuria, no matter what the cell count, is taken as a criterion for a complete renal study, a number of children will be subjected to what is apparently an unnecessary examination, for only 37 per cent of the 64 with pus in the catheterized specimen had demonstrable urinary tract disease. However, as can be noted in Table III, the amount of pus is no guide as to the necessity for cystoscopy. Many of the obstructive lesions are "silent" and a few pus cells the only indication of a pathologic process, so that it is undoubtedly a "greater error to miss making an early diagnosis through neglect of proper examination than to mistake the indication and subject the child to what is apparently an unnecessary examination."

Many urologic lesions in children are "silent" or have misleading symptoms. Only 9 of the 26 children in the study who were found to have urinary tract disease had subjective

symptoms that were in any way referable to the urinary tract. In only 10 was there any presumption of a urinary lesion. The remaining sixteen were discovered through the routine study of pyuria.

Urinary complications in children with bone and joint tuberculosis are not common, and prolonged immobilization on frames or in casts does not tend to urinary stasis, infection and stone formation. Forty-one of the children examined had bone or joint tuberculosis. In none were tubercle bacilli found in the urine after repeated and careful examination. Only 7 had pus in the urine, and none of these had any demonstrable urinary tract disease.

In determining the type of organisms in 183 urines, the fresh smear and cultures agreed in 170, or 92 per cent.

#### **MONONUCLEOSIS, Infectious, Davidsohn, I. Am J Dis Child 49 1222, 1935**

Davidsohn describes a rapid method for the heterophile antibody test which may be read in four hours.

The serum dilutions range from 1:25 to 1:5,120, or higher if necessary. The quantity of the dilution used is 0.25 cc. One-tenth cubic centimeter of a 2 per cent suspension of sheep cells is added. Narrow test tubes, measuring 75 mm in length and 12 mm in the external diameter, are used in order to make possible the reading of the results with a microscope. The test tubes are shaken and placed in a water bath at 38° C for one hour and then in an ice box for one hour. At the end of two hours, the test tubes are shaken until the sediment is suspended, and the results interpreted as +++ and ++ are estimated with the naked eye. The result read as + can be fairly accurately estimated with the naked eye, but a finer, more accurate reading is obtained by placing the test tube flat on its side on the stage of the microscope and looking at the cell suspension with the low power of the objective. The titers obtained with the microscope are from one to two dilutions higher than those read with the naked eye.

#### **LYMPHOGRANULOMA INGUINALE, D'Annoy, R., Von Haam, E., and Lichtenstein, L. Am J Path 21 737, 1935**

Seven endemic strains of the virus of lymphogranuloma inguinale have been isolated and transmitted to animals.

Intracerebral inoculation of infectious material produced a typical meningoencephalitis in the common marmoset while the rhesus monkey proved resistant to such inoculations.

The virus could readily be transmitted to white mice, biweekly inoculations allowing upkeep of its maximal virulence.

Brain emulsions of infected monkeys and mice act as excellent stable and sensitive antigens for the specific diagnostic intradermal reaction of Frei.

Twenty-eight per cent of infected guinea pigs showed enlargement of the regional lymph glands with histologic lesions consistent with the disease.

Experiments with sheep, chickens, and frogs indicate that the virus can infect sheep, that its virulence can be preserved in the bins of chickens, and that frogs cannot be infected.

Cultivation of the virus after the method of Tamura was possibly confirmed.

The virus of lymphogranuloma inguinale as encountered endemically in the poorer negro population of Louisiana, shows rather identical behavior concerning animal transmission as the virus strains studied in other parts of the United States and abroad.

#### **HYPOGLYCEMIA in the New Born, Higgons, R. A. Am J Dis Child 50 162, 1935**

Two cases have been reported which appear identical in every respect except in the family history. It is an open question whether the insulin taken by the mother of the patient in the first case had any effect in producing the hypoglycemia in her baby. Certainly there was no exogenous insulin in the second case.

It seems likely that some of the cases of convulsive attacks during the first few days of life which are now considered to result from birth injury may be due to hypoglycemia. The author feels that a reading of the blood sugar should be made in all these cases before

a diagnosis is made. It seems reasonable to suppose that without proper treatment one or both patients might have died owing to a continued fall of the blood sugar level. Babies take food with great difficulty after severe hypoglycemia develops, and without the use of gavage and parenteral administration of sugar the deficiency of sugar in the blood might not be corrected.

**NEPHRITIS, Hemorrhagic, Infection and, Winkenwerder, W. L., McLeod, N., and Baker, M.** Arch. Int. Med. 56: 297, 1935

An analysis of the relationship between infection with *Str. hemolyticus* and hemorrhagic nephritis in a series of 78 cases observed for from one to eight years is reported. Twenty two of the patients are well, 21 are in the latent stage of the disease, 17 are in the progressive stage, and 19 are dead.

Infections, usually of the upper respiratory tract, caused by *Str. hemolyticus* (alpha type, in a few cases), preceded the onset of hemorrhagic nephritis in the great majority of cases in this series.

Pneumococcal pneumonia, rheumatic fever and syphilis were not considered as causes of hemorrhagic nephritis in this series.

*Str. hemolyticus* is apparently related to the progress of nephritis; for the numbers in which it occurred diminished markedly during recovery but persisted during the progressive stages of the disease. In 11 cases, however, hemolytic streptococci were not shown at any time during the course of the disease.

The cases of hemorrhagic nephritis which were preceded by acute infection manifested by both local and constitutional reactions almost always ended in recovery or entered the latent phase; the cases of nephritis associated with chronic infection at the onset almost always became progressive.

The prodromal period between the beginning of infection and the onset of nephritis varied from three to twenty eight days, the most frequent interval was seven and the average was ten and nine tenths days.

A seasonal variation in the onset of hemorrhagic nephritis coincided with months during which respiratory infections are most frequent in Baltimore.

Exacerbations of nephritis in most instances followed streptococcal infections of the upper respiratory tract; these exacerbations occurred more frequently in the latent and in the progressive stages of the disease. The prodromal period between infection and the exacerbation of the nephritis was usually from twenty four to forty eight hours, in contrast to the prodromal period at the onset of nephritis. Exacerbations also occurred without relation to infection, and also after operative procedures.

Streptococcal infections which occurred during the convalescent stage did not prevent recovery from nephritis, and after recovery was established they rarely caused relapses, whereas the "carrier state," with or without recurring evidences of active infection, was characteristic of the progressive stage of hemorrhagic nephritis.

Surgical removal of foci of infection may fail to influence the outcome of hemorrhagic nephritis.

It is suggested that recovery from or progression in hemorrhagic nephritis is related more to the character of the causal infection: a severe acute infection presages a favorable course; a chronic infection, an unfavorable outcome. It is also suggested that patients with acute infection possess the capacity to react to *Str. hemolyticus*, so that some form of resistance to the infection develops, and as a result recovery from nephritis follows and disappearance of the organism occurs. In patients with chronic infection, this capacity to react to streptococcal infection is lacking, a resistant state does not develop, and as a result the organism persists and the nephritis becomes chronic.

**MERCUROCHROME, Action of, on Normal Human Skin and in Infected Wounds, Hill, J. H.** J. A. M. A. 105: 100, 1937.

There is urgent need for standard methods of studying the in vivo action of antiseptics for special uses. In this paper methods are suggested which, on further refinement, might serve as bases for such standards.

In regard to the action of antiseptics applied to the skin, it is shown that

a Under conditions of practical use no antiseptic studied can invariably sterilize heavily infected skin

b Aqueous solutions of antiseptics are not as a rule suitable for preoperative skin sterilization.

c Both the 2 per cent tincture and 2 per cent aqueous mercurochrome solutions are bactericidal and bacteriostatic on human skin. The 2 per cent tincture of mercurochrome is superior to the aqueous solution on the skin, as has been shown previously. Only the tincture has been advocated for preoperative skin sterilization.

d If comparisons are to be made between the bacteriostatic actions of preparations of iodine and mercurochrome on the skin the order of efficacy, according to the results of our experiments, is as follows: first, the 2 per cent tincture of mercurochrome, second, the 7 per cent tincture of iodine, not removed with alcohol, third, the 2 per cent aqueous solution of mercurochrome, fourth, the 7 per cent tincture of iodine, removed with alcohol.

In regard to the use of antiseptics in wounds, it is shown that

a It is improbable that a single application of any known antiseptic will sterilize a heavily infected wound.

b There is evidence that while both the tincture and aqueous solutions of mercurochrome are bacteriostatic in heavily infected wounds, the aqueous solution, under the conditions of the tests, is superior to the tincture of mercurochrome and to the other antiseptics tested, in that it keeps the bacterial count lower and does not interfere with phagocytosis.

#### DIPHTHERIA, Rapid Method for Identification of *B. Diphtheriae*, Brahdy, M. B., Lenarsky, M., Smith, L. W., and Gaffney, C. A. J. A. M. A. 104: 1881, 1935

Sterile cotton swabs are impregnated with undiluted unheated horse serum to which no preservative has been added. The swabs are then squeezed lightly against the sides of the tube to remove any surplus serum. They are removed and lightly heated over a flame to obtain surface coagulation and possibly, to destroy any antibodies in the serum. The swabs are then used to take the nose and throat cultures in the routine manner. Instead of being implanted on a culture medium the swabs are put in dry sterile tubes, placed in the incubator and examined at the end of two and four hours. The physician's vest pocket may serve as an incubator. At the end of the incubation period, smear preparations are made on slides directly from the swab.

#### MENINGITIS, Acute Aseptic, Viets, H. R., and Watts, J. W. J. Nerv. & Mental Dis. 80: 253, 1934

The disease known as acute aseptic meningitis, first clearly described in 1925 by Wallgren, has now found a well marked place in medical literature.

Patients with this disease, described by the authors in 1929, have been reexamined and found to conform with Wallgren's description.

New cases have been added to the authors' series, making a total of fourteen.

Contrary to the authors' opinion expressed in 1929, they now believe that the changes in the cerebrospinal fluid are not exclusively lymphocytic, although essentially so. Clots, also, may be found in the fluid.

The most important point in differential diagnosis between this disease and tuberculous meningitis is the constantly normal sugar and chloride content of the cerebrospinal fluid in acute aseptic meningitis, contrary to the findings in tuberculosis.

The disease should be considered as a clinical entity, as no definite relationship has been shown to exist between it and any other diseases.

The essential characteristics of the syndrome are: acute onset, nonpurulent (lymphocytic) character, inflammation of meninges.

The disease, however, has other characteristics: it is benign, self limited, seldom, or perhaps never, results in late manifestations. It is mildly epidemic, and the cerebrospinal formula is strongly lymphocytic without decrease in the sugar or chloride content.

*Acute aseptic meningitis begins acutely or subacutely.*

The course of the disease varies widely in both severity and duration, although all patients recover within a period of a few weeks. The more marked symptoms, such as coma and convulsions, usually disappear after the first or second withdrawal of cerebrospinal fluid.

Examination of the cerebrospinal fluid is essential to diagnosis. In some instances where the disease is not severe, the changes in the fluid are mild. There is a slight pleocytosis, essentially lymphocytic in formula, without polymorphonuclear leucocytes. The pressure is normal; the protein is slightly increased, but not markedly so; and the sugar and chloride contents remain within the normal limits. Most of the cells in the fluid are small lymphocytes, although occasionally one finds a few large lymphocytes.

In general, the cerebrospinal fluid varies directly with the severity of the illness. In the milder cases the cells are few in number and exclusively lymphocytic; in the more severe cases there is a great increase in cells, up to 600 or 700 per c.mm., and polymorphonuclear leucocytes are frequently found. The presence of these latter cells indicates a severe illness, their reduction in number and final disappearance are coincident with recovery, and a careful recording of them, therefore, is helpful to the prognosis. The most important finding, however, in the cerebrospinal fluid is the constant sugar and chloride content. This is in marked contradistinction to the findings in tuberculous meningitis and is the chief laboratory test which is helpful in the differential diagnosis of the two diseases.

Although the chloride content may be slightly decreased, it never varies much from the normal. The colloidal gold reaction falls in the meningitic zone. The Wassermann reaction is always negative; bacteria are not demonstrated by either smear or culture, and guinea pig inoculation gives negative results.

#### ASCHOFF BODY, Studies On the Myocardial. II Life Cycle, Sites of Predilection, and Relation to Course of Rheumatic Fever, Gross, L., and Ehrlich, J. C. Am. J. Path. 10: 489, 1934.

There has been presented in this report a study of the life cycle of the myocardial Aschoff body, based on an examination of the clinical records and autopsy material from 70 cases that presented Aschoff bodies in the myocardium. It appears that these specific lesions pass through three stages in development. The earliest phases, represented by small cell coronal and reticular Aschoff bodies, have been found to occur up to the fourth week after the onset of the illness. The middle phases, represented by large cell coronal, syncytial coronal, mosaic and large irregular cell polarized Aschoff bodies, have been found to occur between the fourth and thirteenth week after the onset of the illness. The late phases are represented by polarized Aschoff bodies which occur from the ninth to the sixteenth week after the onset of the illness, and subsequently by fibrillar Aschoff bodies which occur after the thirteenth week of the illness.

The earliest types of specific lesions are apparently influenced in their response by the reactivity of the tissue, depending on whether there has or has not been a previous attack of rheumatic fever, and also by the state of the collagen present in the interstices between the myocardial bundles. As a consequence, the evolution of the lesion may follow one of two main courses, determined by the initial lesion. The latter may occur in the form of the reticular or the small cell coronal Aschoff body. The final phases of the life cycle of the Aschoff body are common to both main courses.

Dividing the material into four groups representing different clinical courses, there appears to be some change both in the incidence of the types of Aschoff bodies present in the myocardium and in their localization. The findings reported here, however, can by no means be considered as furnishing sufficient statistical evidence on which to base final conclusions on this point. That the tempo of the life cycle may be considerably faster or slower than what has been described in this report seems very probable. Some of the stages in the "model" of the life cycle presented by the authors may be absent in some cases, abbreviated in others, or indeed, appear in the reverse order from what we have suggested. These facts can be determined with greater accuracy only after examining a much more extensive series of cases and, in the last analysis, must await confirmation by the hitherto unsuccessful transmission of this disease to animals. It is hoped, however, that further studies will be made along these lines in order that some of these interesting relations may be placed on a firmer footing.

## REVIEWS

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Books and Monographs for Review should be sent direct to the Editor,  
Dr Warren T Vaughan, Professional Building, Richmond, Va

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### Food and Beverage Analyses\*

**A**LTHOUGH small and compact, this volume contains a remarkably comprehensive survey of foods, including those canned and packaged, somewhat of an innovation in texts of this character

Not only the nutritive and caloric values of foods and beverages are tabulated but also their mineral, iodine and vitamin content The alcoholic table is as of 1935

In each instance, not only the percentage proportion of the various elements, but also their content in grams per each portion of a specific food is given

This volume should be of great value to all those interested in nutrition and home economics and, of course, especially to those interested in the preparation of special diets for specific purposes

It may be regarded as an authoritative text, perhaps the most comprehensive yet available

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### Clinical Parasitology and Tropical Medicine†

**T**HE purpose of this volume is to present a survey of the subjects, which, while not all inclusive, will nevertheless discuss in an adequate manner the present status of this specialized field of medicine

The major divisions of the text consider Part I General Considerations of Etiology and Pathology, environmental conditions, laboratory diagnosis, and treatment Part II discusses the diseases due to protozoa, dysenteries, flagellate diarrhea, malaria, trypanosomiasis, leishmaniasis, and spirochetosis and trepanemiasis Part III is concerned with the diseases caused by metazoa nematodes, trematodes, and cestodes, Part IV discusses the bacterial diseases most common to the tropics, Part V Diseases of Undetermined Etiology under which are included filterable viruses, metabolic disturbances, and vitamin deficiencies, while Part VI concerns a discussion of climatic diseases and animal poisons, including heat stroke and poisonous bites and stings

It will be seen that the authors have set themselves a difficult task There is much in the book that is useful, especially, perhaps, as concerns laboratory diagnosis The specialist in this field, however, will find the text somewhat less than his requirements demand, while the student will not be in a position either to note or estimate the importance of that which is omitted or touched upon somewhat briefly

The sections on treatment describe the authors' methods at some length, others receiving but brief mention in many instances, and in some cases being omitted entirely

There are some typographical errors, principally in the spelling of names, etc., which require correction, these, together with the factors already noted, will be corrected in future revisions

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\*Food and Beverage Analyses By Milton A Bridges M.D. Director of Medicine Department of Correction Hospitals New York Assistant Professor of Medicine and Lecturer in Therapeutics New York Post Graduate Medical School etc. Cloth 248 pages Lea and Febiger Philadelphia Pa

†Clinical Parasitology and Tropical Medicine By Damaso de Rivas M.D. Professor of Parasitology Graduate School of Medicine University of Pennsylvania etc and Carlos T de Rivas M.D. Pathologist Santa Toma Hospital Panama Cloth 361 pages Figs 144 1 colored plate Lea and Febiger Philadelphia Pa

### The Design of Experiments\*

THIS is a companion volume to the author's previous work "Statistical Methods for Research Workers" and to the general body of scientific workers will be found of equal interest and value.

### Demonstrations of Physical Signs in Clinical Surgery†

HERE, without doubt, is an excellent book which should be in the hands of every physician.

That an accurate diagnosis is the essential prerequisite to intelligent treatment is obvious, just as it is equally obvious that careful and thorough examination is essential to the formation of a diagnosis. But it may sometimes be questioned if physical examinations are always as careful and thorough as they might be or if all physicians bring to them the many "tricks of the trade," so to speak, which have evolved from experience.

Despite the fact that the title might suggest that the book is primarily of value to the surgeon, it is, on the contrary, an extremely valuable book for the physician in general. For, in many instances, the conditions ultimately referred to the care of the surgeon are first seen and must first be recognized by the physician.

The book is obviously built upon a varied and extensive experience well and ably presented to the reader.

The style is clear, there is no ambiguity, and the author speaks clearly and simply. The illustrations are not only numerous and well reproduced but they illustrate clearly.

This volume can be recommended without reserve as one well fitted for the physician at large to use as a desk companion, frequent reference to which will greatly benefit both doctor and patient.

It is among the best of all the books of its kind which this reviewer has encountered.

### Tumors of the Urinary Bladder‡

IN THIS excellent monograph Dr. Beer reviews the subject of tumors of the urinary bladder based upon an experience covering 650 cases encountered during the last 25 years, 388 being definitely malignant tumors and 262 definitely benign. In this series males were more frequently attacked than females, the ratio being 4.5 to 1. The present consensus of urologists is in favor of cystoscopic high frequency electrocoagulation in the treatment of benign bladder tumors, while surgery is still the choice of the majority for malignant cases.

Beer believes that surgery, despite its higher mortality, is still the more promising method of attack and offers the better chance of permanent cure. He practices total excision and hopes that improvement in technic may eventually lead to decreased mortality.

This little volume is evidently the fruit of varied and extensive experience and may well be read with interest and profit by all who are interested in this subject.

Well printed and excellently illustrated it is a contribution of distinct value.

If it presents the experience of but one clinic, it is an experience which has been, not only extensive, but carefully recorded and analyzed.

There is a bibliography of 27 pages and also a satisfactory index.

\*The Design of Experiments. By R. A. Fisher, Golton Professor, University of London. Cloth, 252 pages, Oliver and Boyd, Edinburgh, Scotland and London.

†Demonstrations of Physical Signs in Clinical Surgery. By Hamilton Bailey, F.R.C.S., Surgeon, Royal Northern Hospital, London, etc. Cloth, ed. 5, 287 pages, 341 illustrations and colored plates. William Wood & Co., Baltimore, Md.

‡Tumors of the Urinary Bladder. By Edwin Beer, M.D., Visiting Surgeon, Mount Sinai Hospital, New York. Cloth, pp. 166, 52 illustrations, 8 in color. Wm. Wood & Co., Baltimore, Md.

# *The Journal of Laboratory and Clinical Medicine*

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## *The American Association for the Study and Control of Rheumatic Diseases*

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### INTRODUCTORY REMARKS\*

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ERNEST E. IRONS, M.D., PRESIDENT, CHICAGO, ILL

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WE TAKE pleasure in welcoming all to this Second Annual Meeting of the American Association for the Study of Rheumatic Diseases.

The contributions to last year's program were extremely interesting and valuable. They have been collected, printed and distributed to members and others. The papers on this program give promise of being equally important and profitable. It is the plan of your Executive Committee to publish these Proceedings.

You will note by the program that this morning session is devoted to chronic arthritis and the afternoon to rheumatic fever. We are specially gratified by the excellence of the program prepared by the Committee for this afternoon.

It is evident that the study of the complex system of variables presented in arthritis must be conducted by a consideration of each one or of each group of these variables and so of necessity studies of arthritis are concerned with one or another of the causative agents or contributing elements in the disease. Such studies are frequently unrelated except for the fact that they are part of the investigations necessary to the ultimate understanding of the larger whole.

Even before the results of these separate studies are completely correlated, they are seen to follow certain general lines. It is to these current

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\*The articles contained in this issue are papers read at the Second Annual Meeting of The American Association for the Study and Control of Rheumatic Diseases and the Fourth Conference on Rheumatic Diseases June 10-13, 1935, Atlantic City, N. J. The papers on Chronic Arthritis read at the first session of the meeting appear in this issue. The remainder of the proceedings, articles on Rheumatic Fever will appear in the March issue of the Journal.



trends of thought in arthritis that I wish to refer. Most of these comments are so familiar that reference to them almost calls for an apology. Nevertheless a restatement of facts is sometimes profitable.

The concept of chronic arthritis as a general disease rather than one of the joints alone is steadily gaining ground. It is generally conceded that there are many initiating and contributing causes in chronic arthritis. These include infection, trauma, age, heredity, disturbances of vascular supply or of general and local nutrition, and metabolic and glandular dysfunction. Various combinations of these causes and conditions in individual patients result in varying clinical pictures, whose common factor is involvement of joints and structures attached to the bony skeleton. No one single cause or condition is common to all cases of arthritis.

It seems generally agreed that (no matter how great the disagreement as to the original cause of first symptoms) the division of chronic arthritis into two great groups, the atrophic (rheumatoid) and hypertrophic (osteo-) arthritis, is extremely serviceable. One of the chief values of this classification rests on the fact that it takes into consideration the previous reactions of the tissues of the patient, and so prophetically indicates the kind of changes he is likely to suffer in the future, and therefore the measures which should be taken to meet and modify the disability which threatens him.

There still is disagreement as to whether these two great groups represent separate clinical entities. Some hold that hypertrophic arthritis is an entity, no matter what may be concluded as to the atrophic forms, and still others are firm in support of the clinical entity of the atrophic group. It seems to be generally agreed however that evidences of the effects of infection are much more clear in atrophic, than in hypertrophic arthritis.

Is it not possible that the disease process in chronic arthritis assumes this or that course with the production of one or another type of arthritis by reason of the kind, age, and resistance of tissues of the patient whose joints have suffered an initial insult? It may even be argued that the same insult which initiates in one person an atrophic arthritis might, if received by a person whose tissues were less vulnerable, have caused no more than a temporary disability. In this connection consider the variety of types of arthritis which may follow gonococcal infection, from an acute temporary and quickly healing arthritis to the chronic forms indistinguishable from "atrophic arthritis," or again from spondylitis ankylopoietica of the British classification.

The influence of heredity seems as clear in atrophic arthritis occurring in grandmother, mother, and daughter, as in the family incidence of early hypertension. Racial, familial, and individual peculiarities in connective tissue response to injury as illustrated by the variable formation of keloid in surgical scars, and the varying rapidity and extent of callus formation after fractures, indicate the wide range of tissue response to trauma. The same factors may influence the course of the healing of tissues of joints.

Other similar comments might be offered with respect to the progress of disease in the joints themselves, as well as other perhaps obvious observations

on the reactions of the body as a whole to infection, trauma, and local and general derangements of nutrition. While some of these questions are controversial, they serve to reemphasize the importance to the student of arthritis of the conception of arthritis as a disease of the entire body.

Another phase of the problem of arthritis concerns the use of physiotherapeutic measures. In hospital centers where the study of chronic arthritis is particularly advanced, equipment of many sorts for hydrotherapy and physiotherapy has been installed. Some of this is of undoubted value, some suggests an associated and undesirable commercialism. Much of it is expensive, and in the form offered is not practicable for home treatment.

While many patients are treated in larger centers, and many more come for diagnosis and the outlining of a program to be carried out later, the majority of sufferers from chronic arthritis must be treated entirely in their own communities by their family physicians. One of the greatest services to these patients will be the formulation of suitable methods of treatment in the home by the use of equipment improvised at very small expense. A fuller knowledge by physicians of what can be done, and how it should be done, will benefit patients and at the same time lessen the excuse for the existence of pseudomedical cults.

Before leaving the subject of physical therapy, I wish to suggest the possible good results to be obtained by the application of negative and positive pressure to the joints of the extremities by means of the Paevex or similar machines now used in the treatment of vascular obstruction and threatened gangrene of the extremities.

Certain cases of atrophic arthritis with impaired nutrition and shiny atrophic skin will be called to mind as possibly suitable for this type of treatment. In a few cases selected for trial, results seem to warrant further experimental employment of this treatment.

Another problem concerns the establishment of hospitals for the treatment of arthritis where study and treatment may be highly developed by well qualified investigators in bacteriologic, metabolic, and orthopedic aspects, skilled in the application of knowledge to the needs of each individual patient.

Such clinics already exist at certain medical centers and will be most successful if developed as a part of a large general clinic. Clinics for arthritis alone, established apart from other medical projects, may offer superior custodial advantages for those patients whose hospital care must be prolonged over many months. They are likely, however, to suffer from lack of stimulus from other departments of a general clinic. Undoubtedly there may be instances in which a separate institution for the care of arthritis would do better work than a general hospital by reason of the centering of effort on one subject under the inspiring leadership of some one man.

On the other hand, the relations of arthritis to other diseases might thus tend to be minimized, and it would seem that the chances of reversion from active centers of study to institutions for custodial care would be greater if the arthritis hospital were detached than if it were closely associated with a general medical clinic.

## ACCELERATING FACTORS IN CHRONIC HYPERTROPHIC ARTHRITIS (OSTEOARTHRITIS)

RUSSELL L. HADEN, M.D., AND WIRT A. WARREN, M.D., CLEVELAND, OHIO

**H**YPERTROPHY of bone may develop secondarily around a joint in almost any type of chronic arthritis. Such a change is common in the late stage of chronic atrophic (rheumatoid) arthritis. Abnormal mechanical stress on a joint otherwise normal also may result in a hypertrophy of subchondral bone as in scoliosis. Primary hypertrophic arthritis (osteoarthritis), however, represents a degeneration of joint cartilage and proliferation of subchondral bone incident to physiologic aging and the wear and tear of continued use. The fundamental pathologic process is a fibrillation of cartilage, softening and erosion of the joint surface, and a loss of the protective power normally afforded by the cartilage. Any factor which adds to the physical stress of the joint will hasten erosion of cartilage after softening has begun. The more important physical factors are occupation, static deformities, and trauma. Such factors, however, are not etiologically related to the primary degeneration in the cartilage.

If degeneration of cartilage in primary hypertrophic arthritis (osteoarthritis) represents an aging process, we should not think of etiologic factors here since old age is a normal stage in the life of all body tissues. Old age, however, becomes a disease if it comes prematurely as a result of the shortening of the normal span of life by acceleration of the chemical processes on which life depends. There are certain physiologic criteria corresponding to different chronologic ages. The physiologic and chronologic ages may be dissociated, more commonly with the physiologic exceeding the chronologic age. Studies show that every person will have some degree of degeneration of cartilage and hypertrophy of bone if he lives long enough, just as he will have gray hair. In clinical work, our problem is the frequent development of such changes prematurely or a physiologic age exceeding the chronologic age. Numerous factors may contribute to or accelerate physiologic aging. The problem is much the same as premature graying of hair and often runs parallel with it. If one enumerates the factors which may cause gray hair prematurely, he has in mind the factors which lead to degeneration of joint cartilage out of proportion to age.

We have analyzed fifty consecutive cases (8 males, 42 females) of chronic hypertrophic arthritis in relation to the following accelerating factors: (1) Heredity, (2) worry and emotional stress resulting in lack of rest, (3) hard physical labor, (4) illness of general nature from any cause, (5) focal infection, (6) gastrointestinal disorders with disturbance in alimentation, (7) metabolic abnormality, especially lowered metabolic rate, (8) endocrine imbalance, and (9) disturbed circulation, especially by hypertension and arteriosclerosis. Each patient came in primarily because of joint symptoms and was found to have an active arthritis which was classified as degenerative in type. We have

included no cases in which degenerative arthritis was an incidental finding. The method of study after a careful history and complete physical examination has been as follows: (1) Roentgenogram of a typical joint, (2) routine study of blood for anemia and of urine for any abnormality, (3) search for a focus of infection—teeth, tonsils, sinuses, prostate and cervix, (4) complete study of gastrointestinal tract (gallbladder, size and shape of colon, gastric acidity), (5) sedimentation rate of erythrocytes, (6) blood chemical study (glucose tolerance, ure acid), and (7) basal metabolic rate.

A few patients did not have every examination. The glucose tolerance test was the one most frequently omitted. The group of 50 includes 8 males and 42 females. The average age of the males was fifty-two and of the females, fifty-one. The age range of the males was from thirty-nine to fifty-eight and of the females, from thirty-four to sixty-nine years. The average duration of symptoms in the males was seven plus years and in the females four plus. Certain important factors are most difficult to evaluate statistically. Heredity is a most important factor, but a family history of arthritis is so unreliable that no figures were attempted for this factor. The condition of any tissue at any time necessarily depends on how good it was to start with, so heredity is fundamental. It is also difficult to evaluate worry and emotional stress as well as physical labor, but exhaustion and fatigability were prominent symptoms, however, in addition to the joint disability.

Only two patients gave a history of a serious general illness within the preceding five years. Most were remarkably well except for the exhaustion and joint disability, although a few were of the chronic invalid type. When the age group is taken into consideration a relatively small proportion (42 per cent) had evidence of focal infection. Thirty-two per cent showed chronic tonsillar infection, 20 per cent had some dental infection, and 10 per cent had other foci such as chronic prostatitis.

The incidence of overweight is striking. Sixty-two per cent of the group weighed more than normal for their age and height. The average percentage of overweight was twenty-five pounds. This increased weight adds to the physical strain of weight bearing and indicates a metabolic overstrain as well, since no patient who is gaining weight is in metabolic balance.

The tendency to overweight is closely correlated with a lowered rate of metabolism. Eighty-four per cent of the group had a basal metabolic rate below the normal average for their age. The average decrease below normal was 15 per cent. The rates in a small number were above normal (16 per cent) showing only a slight increase (5 per cent). Most patients had only a single determination, and since the rate is often higher than it should be on the first test, it is probable that a still larger number actually had a low metabolic rate. Since the metabolic rate records the speed at which the tissues are working, these results indicate a marked slowing of chemical activity. Old age after all is only a gradual slowing up of metabolic processes, so the characteristic low metabolic rate is the best indication of premature aging in chronic degenerative (hypertrophic) arthritis (osteoarthritis).

The sedimentation rate of the erythrocytes was within normal limits in 80 per cent of the cases and very little elevated when beyond the normal range. Not infrequently, an increased suspension stability was observed associated with a rate lower than the average normal. This only verifies the observations of other workers. Anemia was a rare finding. Only 4 per cent of the total had a hemoglobin below 75 per cent (11.5 gm. per 100 c.c.). The uric acid constantly tended to be elevated although not markedly so. Fifty-five per cent had a uric acid of more than 2 mg. (but not markedly so) which is above our average with the method used. The single blood sugar determinations were seldom above normal, but 22 per cent of the group showed a diminished tolerance or a frankly diabetic curve with a glucose tolerance test.

In the study of the gastrointestinal tract, 60 per cent were constipated. It is difficult to evaluate the importance of this finding when it is so common. No attempt was made to procure a dietary history since a dependable one is so difficult to obtain. Overeating, both in amounts of food and in starchy foods with low intake of protective foods, often seemed evident. Twelve per cent had achlorhydria, 20 per cent hypoachlorhydria and 28 per cent hyperachlorhydria, so the gastric acidity seems of little importance. Gallstones were found in 14 per cent and a nonfunctioning gallbladder by the Graham-Cole test in 80 per cent. The colon was atonic and redundant in 28 per cent. This finding was frequently observed to a high degree.

The association of chronic degenerative arthritis with the menopause has long been emphasized. Only 19 per cent of the 42 females were still menstruating. The onset of symptoms had been coincident with the menopause in 36 per cent, and after the menopause in 45 per cent. No other defects in endocrine activity were discovered except the frequent finding of dry hair and skin and brittle nails, suggestive of hypothyroidism in the patients with hypometabolism.

Vascular disease is also difficult to evaluate statistically, although the arteries are frequently well outlined by calcareous deposits in radiographs of the joints. This is often much out of proportion to what we consider normal in relation to chronologic age. Twenty-four per cent of the females had a systolic blood pressure over 140. None of the males had a systolic pressure over 145. The frequent observation of calcareous deposits in arteries, the common finding of arterial hypertension, and the well-known gradual decrease in capillary bed with age, all emphasize the circulatory factor in chronic hypertrophic arthritis.

#### DISCUSSION

The results of this study only emphasize again certain salient facts concerning degenerative (hypertrophic) arthritis (osteoarthritis) which are already known to most students of chronic arthritis. Infection evidently plays a minimal part in the acceleration of the joint disease. The most significant findings are the tendency to overweight and the great frequency of a low metabolic rate. The patients are nearly all within the age period when degeneration of tissues begins or is prominent. The disease was always slowly progressive, so symptoms had been present for several years before medical aid was sought.

We have been much impressed with the constancy with which the patients complained of exhaustion and the frequent disproportion between chronologic and physiologic age. It seemed evident frequently that the chemical activity of body tissues must have been at low ebb for years in many patients. Normal old age may be thought of as a gradual slowing up of metabolic activity. In the group of conditions so commonly associated with this disease are arterio sclerosis and hypertension, obesity, diabetes, and chronic hypertrophic arthritis. We have a premature slowing of metabolism characteristically in degenerative arthritis. Patients are now being observed who are known to have had a low metabolic rate for several years before the signs of degenerative arthritis were evident. The obesity is commonly due to a low rate of metabolism. It seems possible that toxic substances are formed when the combustion is impaired, although it is equally possible that the important factor may be impaired nutrition of the cartilage secondary to the low metabolic rate or impaired circulation. Unfortunately, little is known about intermediate products of metabolism which may injure cartilage. Uric acid and homogentisic acid are two substances known to deposit in and damage joint surfaces. There must be others.

After the degeneration of joint cartilage has taken place, nothing can be done to replace it. Measures which increase tissue activity will do much to slow the progress of the disease. If accelerating factors are looked for and eliminated, much can be done to decrease the incidence of serious or disabling chronic hypertrophic arthritis (osteoarthritis).

#### SUMMARY

Fifty consecutive patients with chronic hypertrophic arthritis (osteoarthritis) have been studied for factors which influence the development or progress of the disease.

Women predominate in the series—five to one.

The degeneration of cartilage characteristic of the disease is a manifestation of the physiologic aging of tissues.

Increased physical strain will hasten the erosion of the degenerated cartilage.

Certain factors will accelerate the primary changes in the cartilage.

The more important accelerating factors are obesity, lowered metabolic rate, disturbed alimentation, endocrine dysfunction, and circulatory inadequacy.

#### DISCUSSION

DR WALTER A. BAUER, BOSTON.—For the past six years, Dr. G. A. Bennett and I have been interested in this type of joint disease. To obtain an accurate picture of supposedly normal joints at various ages, we collected a series of knee joints from normal individuals who so far as we could determine had never had symptoms or signs of previous joint disease. At least six knee joints representing each decade of life from the first to the ninth, were obtained at necropsy. With each succeeding decade in life beyond the second, the knee joints showed increasing pathologic changes, degenerative in nature and indistinguishable from those characteristic of chronic degenerative arthritis. The frequency and extent of these changes increased so rapidly from the second decade on that by the fifth decade of life all joints showed the characteristic changes of chronic degenerative arthritis.

In any discussion pertaining to the cause of degenerative arthritis one must appreciate two facts: (1) That the pathology in this type of joint disease is primarily a degeneration

of articular cartilage, other changes being secondary to this process; (2) that, except for its limited perichondrial blood supply, articular cartilage is an avascular structure. The fact that articular cartilage has a more limited source of nourishment than most other body tissues is probably sufficient reason for its having a low metabolism and a limited ability to repair. If a tissue has a limited ability to repair, one would expect to find more evidence of the changes resulting from the wear and tear of daily use and increasing age than in other tissues where such limitations of repair do not exist. That such is the case with articular cartilage would seem to be borne out by our studies which with other experimental and clinical observations have led us to conclude that degenerative arthritis is primarily, if not solely, the result of wear and tear, with changes resulting from long-continued use of a relatively avascular structure which has a limited ability to repair. The greater the wear and tear, unusual use and repeated traumas a joint is subjected to, the earlier these changes will appear.

We agree with Dr. Haden that some persons inherit better cartilage than others. Those who inherit poor articular cartilage will develop this type of joint change, at a much earlier age. That such is true seems often evident from the family history of such patients.

There are often other factors present which will accelerate this degenerative process. The known factors operative in the case of the knee joint include obesity, knock knees, bowlegs, flat feet, imperfectly set fractures, faulty body mechanics, etc. Examples of such accelerating factors are constantly seen in the clinic. Loss of pain sense, as in tabes dorsalis, syringomyelia, and leprosy, allows such changes to take place more rapidly unknown to the patient.

Dr. Haden suggests the existence of other accelerating factors, particularly of chemical origin. To date we have no proof of their existence. However, we do occasionally see an individual who develops obvious degenerative joint disease at an earlier age than normal without a satisfactory explanation such as poor inheritance or any obvious accelerating factor. We must study this small group more carefully to determine what factors are at work which could impair the articular cartilage and thus allow for the early development of such joint changes.

DR. M. H. DAWSON, NEW YORK, N. Y.—Dr. Haden has presented us with interesting information on certain metabolic changes which occur in degenerative arthritis. Has he studied the corresponding changes in other degenerative diseases such as arteriosclerosis? From his data it would appear that he is comparing changes observed in degenerative arthritis with findings in normal individuals. Before the significance of his observations can be evaluated properly it will be necessary to carry out corresponding studies in other chronic degenerative diseases.

DR. J. A. KEY, ST. LOUIS, MO.—I wonder about controls. I was impressed with the fact that uric acid determinations were included in the charts. Long before I was born people talked about the relationship of uric acid to arthritis of this type. Sometimes after these old theories are given up some one comes along and finds a basis for them. Some one should study some controls to see if there is basis for the uric acid theory.

DR. RUSSELL L. HADEN, CLEVELAND, O. (Closing).—May I answer Dr. Dawson's question by asking him one? What makes arteriosclerosis? I do not think these findings are limited to degenerative arthritis, since they are found with equal frequency in other degenerative diseases. I cannot give figures for a control group. Heredity, an important factor, is something we can do nothing about. In the group reported here we have tried to find something influencing the development of degenerative arthritis, and to see if we cannot correct the influencing factors. Such an approach is important in all degenerative diseases. Arteriosclerosis, hypertension, obesity, diminished carbohydrate tolerance, and degenerative arthritis frequently occur together. I think we will eventually find chemical changes responsible for all of them.

# BACTERIOLOGIC AND IMMUNOLOGIC STUDIES IN ARTHRITIS\*

## I RESULTS OF BLOOD CULTURES IN DIFFERENT FORMS OF ARTHRITIS

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THE importance of correct classification of cases in studies of joint disease is obvious, but especially so in attempts to evaluate the efficacy of different forms of treatment. Although great advances have been made during the past thirty years in the differentiation of various types of these diseases, much remains to be accomplished, for, while typical examples of rheumatic polyarthritis, rheumatoid arthritis, osteoarthritis, gout, gonococcal arthritis, and others are now easily recognized by clinical and simple laboratory means, there are many atypical examples which can be placed in their proper categories only after long observation or never. The work here reported is part of a general study of methods which might prove of value in the differential diagnosis of such cases.

During recent years there has been a revival of interest in bacteriologic and serologic tests as applied to the study of arthritis. This has come about largely because of three developments: (1) the success of some investigators in obtaining positive blood cultures in certain types, (2) the accumulation of indirect evidence linking hemolytic streptococci with rheumatic fever and rheumatoid arthritis, and (3) the development of new serologic tests for the recognition of hemolytic streptococcal infections. The purpose of this and the following paper is to report results obtained in applying certain of these bacteriologic and serologic methods to the study of a group of patients with various types of arthritis, this first paper is concerned with results of blood cultures.

In view of the availability of comprehensive reviews<sup>1, 2</sup> covering reports of blood cultures in rheumatic fever and chronic arthritis, no detailed historical résumé is required here. In Table I, however, are summarized results obtained in representative investigations since the adoption of Schottmüller's<sup>3</sup> classification of streptococci according to their effect on blood. This list makes no pretense at completeness but serves to illustrate the often emphasized lack of uniformity in results obtained by different workers. Chief interest, from the point of view of the present study, centers in the reports of Cecil, Nicholls and Stainsby<sup>11, 12, 13</sup> because of their high percentages of positive cultures and the uniformity with which they obtained green streptococci from patients with rheumatic fever and hemolytic streptococci from those with rheumatoid arthritis.

### MATERIAL

Blood cultures were included in the routine study of all patients with joint disease entering the wards and Arthritis Clinic of the Third (New York Uni-

\*From the Department of Medicine, New York University College of Medicine and the Third (New York University) Medical Division of Bellevue Hospital.



TABLE I

## SUMMARY OF RESULTS OF BLOOD CULTURES OBTAINED BY VARIOUS INVESTIGATORS

AUTHORS AND DISEASES STUDIED	NUMBER OF PA- TIENTS	PA- TIENTS POSI- TIVE	PATIENTS YIELDING				REMARKS
			STREPTOCOCCI			DIPH- THEROID BACILLI	
			HEMO- LYTIC	GREEN	INDIF- FER- ENT		
		PER CENT*	PER CENT*	PER CENT*	PER CENT*	PER CENT*	
1917 Moon and Edwards <sup>1</sup> Acute arthritis	40	40.0	2.5	32.5		5.0	2% staph. and 5% <i>B. mucosus</i>
Chronic arthritis	83	26.0		22.0		4.0	4% <i>B. mucosus</i>
1917 Swift and Kinsella <sup>5</sup> Rheumatic polyarthritis	58	12.0		12.0			
1920 Richards <sup>6</sup> Chronic arthritis—types?	104	13.0		13.0			
1924 Freund and Berger <sup>7</sup> Rheumatic polyarthritis	23	65.0	4.0	65.0			4% mixed green and hemolytic
Miscellaneous diseases	35	14.0	6.0	14.0			6% mixed green and hemolytic
1927 Hadjopoulos and Burbank <sup>8</sup> Chronic arthritis—types?	145	17.0	6.0	4.0	1.0	5.0	1% strep. (variety?) 1% mixed hemo- lytic and green 3% staphylococci
1928 Lazarus-Barlow <sup>9</sup> Rheumatic fever	33	0.0					
1928 Suranyi and Forro <sup>10</sup> Polyarthritis—type?	25	68.0		68.0			
1929 Cecil, Nicholls, and Stains by <sup>11-13</sup> Rheumatoid arthritis	78	70.0	51.0	8.0	3.0	5.0	3% <i>Micrococcus</i> <i>zymogenes</i>
Rheumatic fever	60	60.0	1.5	55.0	1.5	1.5	
Osteoarthritis	23	0.0					
Normal controls	20	0.0					2% strep.—vari- ety?
Pathologic controls	61	7.0		5.0			
1929 Nye and Seegal <sup>14</sup> Rheumatic fever	25	0.0					
1929 Rosenow <sup>15</sup> Chronic infectious arthritis	19	37.0		37.0			
1930 Jordan and Boland <sup>16</sup> Acute polyarthritis	32	0.0	No streptococci				31% gram-neg.— minute bacilli
Miscellaneous controls	16	0.0	No streptococci				No gram-neg.— minute bacilli
1930 Margolis and Dorsey <sup>17</sup> Chronic infectious arthritis	89	10.0		5.5	3.5	2.0	Both indif. strep. and diphtheroids in 1 culture.

\*Percentages are calculated from figures of authors quoted to make data in this table comparable with those in Table II. Most of the percentages are merely approximate.

†These figures designate numbers and percentages of cultures rather than of patients.

TABLE I—Cont'd

AUTHORS AND DISEASES STUDIED	NUMBER OF PATIENTS	PATIENTS POSITIVE	PATIENTS YIELDING				REMARKS
			STREPTOCOCCI			DIPHTHEROID BACILLI	
			HEMOLYTIC	GREEN	INDIFFERENT		
		PER CENT*	PER CENT*	PER CENT*	PER CENT*	PER CENT*	
1930 Nye and Waxelbaum <sup>18</sup> Rheumatic fever Acute infectious arthritis Chronic infectious arthritis	12 11 10	00 00 100				100	Occasional staphs in most groups
Osteoarthritis Gonococcal arthritis Miscellaneous diseases	5 19	00 00 00					
1931 Coburn <sup>19</sup> Rheumatic fever	54	100	25		70	60	2% more gave staphylococci
1931 Bernhardt and Hench <sup>20</sup> Chronic infectious arthritis	20	50				50	20% more gave staphylococci
1931 Gray and Gowen <sup>21, 22</sup> Rheumatic fever Rheumatoid arthritis	1 200	390 480		380		'Small per cent	
Osteoarthritis	79	00		480 "Similar to Cecil's typical strains"			
1931 Weil <sup>23</sup> Rheumatic polyarthritis	75	80		60			2% streptococci (variety?)
1932 Ashworth <sup>24</sup> Rheumatoid arthritis	18	406	252 streptococci "similar to Cecil's typical strains" 94 diplococci				Staphylococci
1932 Cooley <sup>25</sup> Rheumatic fever Nonfebrile controls	25 10	00 00					Occasional staphylococci in both groups
1932 Dawson, Olmstead & Boots <sup>26</sup> Rheumatoid arthritis	204†	85†		10†	05†	60†	1% gram positive cocci (variety?)
Normal controls	21†	30†				30†	
1932 Lichtman and Gross <sup>27</sup> Rheumatic fever	188	105	05	20	00		15% streptococci (variety?) 05% pneumococcus
Rheumatoid arthritis	48	80	20		40		
Miscellaneous diseases	220	75		35	30		2% streptococci (variety?) 1% streptococci (variety?)
1932 Steinfeld <sup>28</sup> Chronic arthritis—type?	10	200	200				

TABLE I—CONT'D

AUTHORS AND DISEASES STUDIED	NUMBER OF PA-TIENTS	PA-TIENTS POSI-TIVE	PATIENTS YIELDING				REMARKS
			STREPTOCOCCI			DIPH-THERIOID BACILLI	
			HEMO-LYTIC	GREEN	INDIF-FER-ENT		
		PER CENT*	PER CENT*	PER CENT*	PER CENT*	PER CENT*	
1933 Callow <sup>29</sup> Rheumatic fever	367†	53.0†	0.3†	18.0†	3.0†	See re-marks	4% mixed types and 27% pleo-morphic bacilli
Normal controls	43†	14.0†					14% pleomorphic bacilli
1933 Wilson and Edmond <sup>30</sup> Rheumatic fever	236†	22.0†		4.0†	7.0†	See re-marks	11% pleomorphic bacilli
Normal and pathologic controls	153†	29.0†		8.0†	4.0†		17%† pleomorphic bacilli

versity) Medical Division of Bellevue Hospital and the Arthritis Clinic of the New York University College of Medicine Clinic over the period October, 1932, to August, 1935. On discharge from the wards all patients were referred to the clinics to make possible the further observation of those who would cooperate. Including normal individuals and patients with miscellaneous diseases used as controls, 575 blood cultures were taken on 459 persons. Of these arthritic patients, approximately two-thirds were first seen on the wards and one-third were ambulatory cases seen only in the clinics.

#### SUBDIVISION OF CASES

In a study having as its purpose the evaluation of various laboratory procedures as aids in differentiating various types of disease, an exact statement of the criteria by which cases were placed in the various groups is clearly essential. It should be emphasized, too, that only patients with obvious joint disease were included; those presenting neuritis, myositis, bursitis, and the like were excluded when the final analyses of the data were made. The following subdivisions were used, as convenient for the purposes of the investigation; they are not recommended as a perfectly satisfactory classification:

*Rheumatic Fever with Polyarthritides.*—The criteria for placing patients in this group were: (a) characteristic, migratory polyarthritides; (b) temperature of 103° F. or higher; (c) cardiac involvement as shown by precordial pain, prolonged atrioventricular conduction time, significant arrhythmias, or the development of characteristic valvular lesions; (d) characteristic response to amidopyrine; (e) recovery with no residual joint damage.

*Atrophic (Rheumatoid) Arthritis.*—This diagnosis was used in a narrower sense than that usually employed. All patients so listed showed: (a) progressive and recurrent polyarthritides of the type characterized by fusiform swelling of the joints, especially the metacarpophalangeal and proximal interphalangeal

joints of the hands, without overlying redness or periarthritic induration, (b) only partial relief of joint pain following amidopyrine, (c) steadily progressive disease or partial recovery with residual deformity.

*Genococcal Arthritis*—These patients showed (a) clinically characteristic arthritis occurring during the course of, or shortly following, a known gonococcal infection, or (b) similar arthritis without a history of recent gonococcal infection, but in patients with urethral or cervical smears showing typical gram negative diplococci, no relief of pain following amidopyrine.

*Hemolytic Streptococcal Arthritis*—Of the three patients in this group, two had typical brawny arthritis of the left shoulder during the course of known hemolytic streptococcal septicemia\* and the third had clinically similar arthritis of the left knee containing frank pus from which hemolytic streptococci were repeatedly obtained in pure culture.

*Nonspecific, Low grade Pyogenic Arthritis*—These patients all had brawny induration of one or two joints which had the appearance of low grade, pyogenic infections. In general they may be described as similar to the indurated type of gonococcal arthritis but were without any direct or indirect evidence of gonococcal infection. By many observers in this country these cases are included under the term atrophic (rheumatoid) arthritis but to us it seemed wise to list them separately on purely clinical grounds, at least for the purpose of these studies.

*Hypertrophic (Osteo) Arthritis*—(a) Cases of clinically characteristic arthritis of insidious onset in patients past middle age, (b) hypertrophic bony changes on roentgenographic examination, (c) erythrocyte sedimentation rates within normal limits or only very slightly above.

*Miscellaneous Arthritis*—Included in this group were nine patients with spondylitis, four with gout, two with sciatica, and one each with tuberculous arthritis, Charcot's joint, Still's disease, traumatic arthritis, meningococcal arthritis, arthritis accompanying ulcerative colitis of unknown etiology, and arthritis accompanying amebiasis. The patients with spondylitis had involvement of no other joints; three were probably of osteoarthritic origin while the remaining six were of the type usually considered a subdivision of rheumatoid arthritis.

*Unclassified Arthritis*—Almost one fifth of the arthritic cases failed to fulfill the criteria as listed and, hence, were placed in an unclassified group. In some instances the nature of the joint disease was entirely unknown and such cases were listed as *wholly unclassified*. Most, however, were obviously of infectious origin although the type was not clear, and they were therefore listed as *unclassified infectious*. In many instances these cases could have been placed in one of the fixed categories with fair assurance, but since the diagnosis was not certain it was considered more accurate to segregate them. Included in the group of unclassified infectious cases were four patients with well defined heart disease of the rheumatic type but with joint changes characteristic of rheumatoid arthritis.

\*Hemolytic streptococci repeatedly grown in pure culture from the blood not only in broth but also in poured agar plates.

In addition to the subdivisions of patients with arthritis, five groups of controls were included as follows:

*Normal Controls.*—All normal individuals were members of the staff or medical students. Each was questioned concerning his recent health and those with illnesses during the preceding month were excluded.

*Senescent Nonfebrile States.*—A small group of patients with generalized arteriosclerosis, old hemiplegias and other degenerative diseases unaccompanied by fever was included. All were elderly and all were more or less cachectic.

*Tonsillitis.*—Only patients with typical acute tonsillitis with high fever, chills, tender regional lymph nodes, and red, swollen tonsils dotted with exudate were included. Throat cultures showed all to be caused by hemolytic streptococci. All blood cultures were taken within twenty-four hours of the temperature peak.

*Other Hemolytic Streptococcus Diseases.*—In this small group there were chiefly patients with puerperal or postabortal infections, erysipelas, and scarlet fever.

*Active Rheumatic Carditis and Chorea.*—Only patients with active disease but without polyarthritis were placed here.

#### TECHNIC OF BLOOD CULTURES

In order to make the work as objective as possible, the cultures were known only by number to the one (R.C.A.) who carried out all bacteriologic procedures subsequent to the venipuncture and inoculation of the primary cultures. Thus, the possibility that certain cultures might unconsciously be given more care than those of normal controls was excluded.

All initial cultures were taken soon after the patient came under observation and before the administration of antirheumatic drugs. Cultures were repeated, as a rule, only if relapses occurred. Thirty cubic centimeters or more of blood were withdrawn in carefully sterilized syringes. The area of skin to be punctured was painted with iodine which was allowed to dry and was then removed with alcohol; after the tourniquet was fastened a second application of iodine was allowed to dry and was not washed off until after the completion of the venipuncture. Covered with a sterile towel, the syringe was then carried directly to a bacteriologic laboratory where the media could be inoculated in suitable surroundings and with the aid of a Bunsen burner.

The first 152 cultures (Oct., 1932, to Jan., 1934) were done according to the aerobic method used by Callow.<sup>29</sup> Her technic was followed exactly even to the extent that all media were prepared by the same department which had made hers.\* Five cubic centimeters of blood were inoculated into each of two bottles containing "double beef infusion broth." From one of these, subcultures were made after forty-eight hours and thereafter once weekly, into four tubes of media: "double beef infusion broth," dextrose (0.5 per cent) phosphate broth, "cooked meat beef heart broth," and phosphate (0.2 per cent) broth. After forty-eight hours these subcultures were examined in stained

\*The authors are indebted to Dr. W. H. Park through whose kindness the media were obtained from the Department of Bacteriology, and to Miss Pauline Epstein and her associates who prepared them.

films and streaked on "double beef infusion" blood agar plates. The second bottle was not opened until the fourth week unless the first showed growth earlier. All original cultures were kept two months and were then centrifuged and the sediment examined in stained films and streaked on blood agar plates prior to being discarded. Further details of technique and the method of preparation of the media can be found in Callow's paper.<sup>20</sup>

The next sixty cultures (Jan, 1934 to June, 1934) were done in duplicate by two methods: the first that just described and the second a modification of that recommended by Gray and Gowen<sup>21</sup> which is in turn a modification of the technique of Cecil, Nicholls and Stainsby.<sup>11</sup> By this method, 30 cc of blood were allowed to clot and the serum was removed. The clot was then broken up under sterile conditions and was transferred to a bottle containing 50 cc of broth prepared as described by Gray and Gowen. Subcultures were made after forty-eight hours and subsequently to the four media used for the cultures of whole blood.

Because the second method gave a somewhat higher percentage of positive results in these sixty patients and because it freed the serum from an additional 10 cc of blood for use in the serologic part of the study, the method using whole blood was discontinued and all remaining cultures were done by the clot method alone.

### RESULTS

An analysis of the results obtained in the entire series is given in Table II, which is, for the most part, self explanatory. In the table all results are shown

TABLE II

RESULTS OF BLOOD CULTURES IN PATIENTS WITH VARIOUS TYPES OF ARTHRITIS AND CONTROLS

	NUMBER OF CULTURES	NUMBER OF PATIENTS	PATIENTS POSITIVE	PATIENTS SHOWING			
				HEMOLYTIC STREP	GREEN STREPT	INDIFFERENT STREP	DIPH THEROID BACILLI
				PER CENT	PER CENT	PER CENT	PER CENT
Normal controls	15	44	5		5		
Senescent nonfebrile states	9	8	25		25		
Tonsillitis (hemolytic streptococcal)	74	61	51	31	15	1	2
Other hemolytic streptococcal diseases	16	14	71	43	22		6
Active rheumatic carditis and chorea	27	22	18	4	14		
Rheumatic fever with polyarthritides	109	90	16	3	12	1	
Atrophic (rheumatoid) arthritis	48	35	19	3	9	5	3
Gonococcal arthritis	46	33	15	9*	6		
Hemolytic streptococcal arthritis	3	3	100	100			
Nonspecific, low grade pyogenic arthritis	27	22	5		5		
Osteoarthritis	46	39	10		10		
Miscellaneous arthritis (gout, spondylitis, tuberculous, etc.)	29	22	15	5	10		
Unclassified arthritis							
Unclassified infectious arthritis	57	40	11	5	3		3
Wholly unclassified arthritis	39	28	14		6	4	4
Total	553	459					

Percentages are shown as the nearest whole numbers.

\*One of these 3 patients had terminal mixed gonococcal and hemolytic streptococcal septicemia.

on a percentage basis; this was done for the sake of easy comparison of results in the different subdivisions even though some of the latter contained too few patients for percentages to be significant. The almost uniformly negative cultures of the normal individuals (2 positive of 44 persons) was in marked contrast to the high percentages of positive results in the patients with purulent hemolytic streptococcal arthritis, acute tonsillitis, and other known hemolytic streptococcal diseases. The next highest figure was obtained in the patients with nonfebrile, senescent diseases, but the group was too small for this unexpected result to be significant. With the exception of the twenty-two patients with nonspecific, low-grade, pyogenic arthritis, of whom only one gave a positive blood culture, the results in the remaining subdivisions were strikingly uniform, not only as to totals but also as to the nature of the microorganisms isolated.

Although all cultures were kept sixty days, the value of such long incubation was questionable. Of the 575 cultures, 138 were positive and of these 80 showed growth within forty-eight hours, 109 within one week, and 122 within two weeks. Of the remaining 16 cultures which were positive after longer incubation, only one gave a hemolytic streptococcus, the others being green and indifferent streptococci and diphtheroid bacilli. In four instances, only one of the two bottles was positive and in these, no growth was obtained until the bottles had been opened several times.

In eighty patients, more than one blood culture was taken, and of these twelve gave positive results. However, in only four instances was more than one culture positive for a given patient, and in one of these the microorganisms isolated at the two times differed in their effect upon red blood cells.

All streptococci were carefully studied before they were subdivided into hemolytic, green, and indifferent groups. This was particularly true in the case of those isolated from patients with rheumatoid arthritis because of the finding of Cecil, Nicholls and Stainsby<sup>11</sup> that their "typical strains" obtained from patients with that disease appeared to be green at first although they proved eventually to be hemolytic. When any question arose, strains were extracted chemically and were tested against known hemolytic streptococcal (Group A) antiserum by the method of Lancefield.<sup>21</sup>

Other than the microorganisms listed in Table II, staphylococci were found in twenty instances, gram-negative bacilli in six, and gram-positive bacilli in six. These occurred with approximately equal frequency in the various groups and were considered contaminants. Diphtheroid bacilli were listed in the table because of the frequency with which they have been considered significant in other studies. Among the patients with gonococcal arthritis, the gonococcus was obtained at blood culture in only one instance and this patient had frank gonococcal septicemia with mixed hemolytic streptococcal septicemia as a terminal event.

In Table II, as stated, the average results are shown for the entire series of cultures taken during the period October, 1932, to August, 1935. Toward the latter part of the study, however, it appeared that the percentage of positive cultures was decreasing. To test this impression a comparison was made

of results obtained with the clot method during the seven month period from the time this method was begun in January, 1934, to August, 1934 and during the ten month period from October 1934 to August, 1935, the dividing line between the two periods being the summer months when work was temporarily discontinued. This comparison revealed the striking difference in results recorded in Table III.

#### DISCUSSION

The explanation of the divergent results illustrated in Table III is far from clear. The two series of cultures were small for statistical comparison yet the differences noted were so consistent between the various subdivisions of the two series that pure chance seems an unlikely explanation. Each series covered approximately the same seasons of the year and the severity of the diseases in each was comparable. Furthermore no changes had been made in technique and all the media had been prepared by the same department throughout. Other explanations naturally suggest themselves, such as the possibility that the higher figures of the first series were due to the introduction of streptococci as contaminants or that the lower figures of the second series were caused by some technical error such as faulty media. Whatever the explanation, the lack of uniformity in results at different periods of this study is in keeping with the even greater differences between results obtained by various investigators as shown in Table I. In spite of the possible faults of the data presented, certain of the findings recorded in Table II are worthy of comment.

Certainly the results do not support the view that streptococci can be isolated from the blood in rheumatic fever and rheumatoid arthritis more frequently than in miscellaneous diseases. Neither do they bear out the theory that strains obtained from patients with these two diseases differ and that hemolytic streptococci predominate in rheumatoid arthritis. Hemolytic streptococci were only rarely isolated in this disease, yet the results in patients with known hemolytic streptococcal infections showed that the cultural methods were adequate for the isolation of these microorganisms. The results obtained in these subdivisions are of greater significance when it is recalled that cultures were known only by number throughout the bacteriologic procedures.

The success with which positive cultures were obtained in cases of tonsillitis was surprising in view of negative results of other workers. Callow<sup>20</sup> reported positive cultures in 66 per cent of eighteen patients with tonsillitis, but green streptococci and pleomorphic bacilli were the only microorganisms isolated. In the present series of sixty one patients, on the other hand, hemolytic streptococci predominated and the frequency with which green streptococci were isolated was comparable to that noted in the other diseases studied. If borne out by future investigations these results will confirm the generally accepted belief that bacteremia exists early in tonsillitis. Further work is in progress to check these results and to study the relationship between strains isolated from the throat and blood of individual patients.

The almost uniformly negative cultures in patients with joint disease of the type referred to as nonspecific, low grade pyogenic arthritis, were surprising



TABLE III  
COMPARISON OF RESULTS OF BLOOD CULTURES DONE BY CLOT METHOD DURING TWO DIFFERENT PERIODS

	CULTURES TAKEN FROM JANUARY, 1934, TO AUGUST, 1934						CULTURES TAKEN FROM OCTOBER, 1934, TO AUGUST, 1935					
	NO. OF CULTURES	NO. OF PATIENTS	PATIENTS POSITIVE PER CENT	PATIENTS SHOWING			NO. OF CULTURES	NO. OF PATIENTS	PATIENTS POSITIVE PER CENT	PATIENTS SHOWING		
				HEMOL. STREPT. PER CENT	GREEN STREPT. PER CENT	INDIFF. STREPT. PER CENT				HEMOL. STREPT. PER CENT	GREEN STREPT. PER CENT	INDIFF. STREPT. PER CENT
Normal controls	10	10	20	0	20	0	27	26	0	0	0	0
Tonsillitis	9	9	55	22	33	0	10	10	20	20	0	0
Rheumatic fever with polyarthritis	15	13	30	7	23	0	51	38	5	0	5	0
Atrophic (rheumatoid) arthritis	23	19	25	5	10	10	19	13	7	0	7	0
Gonococcal arthritis	19	15	27	20*	7	0	23	19	0	0	0	0
Nonspecific, low-grade pyogenic arthritis	9	8	0	0	0	0	13	10	0	0	0	0
Hypertrophic (osteo-) arthritis	16	16	19	0	19	0	23	22	5	0	5	0
Totals	101	90					166	138				

\*One patient had terminal mixed gonococcal and hemolytic streptococcal septicemia.

It had been the authors' expectation that these cases, sometimes called "focus type" or "metastatic" arthritis, would be associated with positive blood cultures more often than other types, since it is usually thought that the joint infection arises from bacteria carried by the blood from some distant focus of infection. Certainly the findings in this small group of 22 patients do not indicate that bacteremia exists in these cases, at least after the development of the joint infection.

Finally, the results obtained suggest that, if large quantities of blood and suitable methods are used, streptococci can occasionally be isolated from the blood of even normal persons, and that in individuals whose resistance has been lowered by illness, the percentage of positive cultures is increased. As noted by Lichtman and Gross,<sup>8</sup> Callow,<sup>9</sup> and others, these results do not suggest that the microorganisms isolated from patients with rheumatic fever and atrophic (rheumatoid) arthritis were the cause of the diseases in those patients, in view of the fact that similar results were obtained in diseases known to have other causes.

#### SUMMARY

As part of a bacteriologic and serologic study of arthritis, blood cultures were done on 310 patients with various types of joint disease and 149 controls. A clot method freed serum for use in serologic work but was not otherwise superior to one using whole blood. Prolonged incubation increased the percentage of positive cultures only slightly over that obtained by incubation for two weeks. Hemolytic streptococci were isolated frequently in tonsillitis and other known hemolytic streptococcal infections. Positive cultures were obtained in 10 to 19 per cent of patients with rheumatic fever, atrophic (rheumatoid) arthritis, gonococcal arthritis and hypertrophic (osteo) arthritis, the microorganisms obtained were chiefly green streptococci and less frequently hemolytic and indifferent streptococci and diphtheroid bacilli. The results did not suggest that the microorganisms isolated were the cause of arthritis in the patients from whom they were obtained. With the exception of the small group of patients with hemolytic streptococcal arthritis associated with frank septicemia, blood cultures were of no value in differentiating the various forms of arthritis studied.

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## BACTERIOLOGIC AND IMMUNOLOGIC STUDIES IN ARTHRITIS\*

### II RESULTS OF VARIOUS IMMUNOLOGIC TESTS IN DIFFERENT FORMS OF ARTHRITIS

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IN THE preceding paper<sup>1</sup> are given the results of blood cultures in different forms of arthritis done as part of a general study of methods which might aid in the differentiation of these diseases. As part of the same investigation, certain immunologic tests were included in the routine study of all arthritic patients, and it is the purpose of the present paper to report the results obtained. These procedures, all of which had been developed and previously applied to the study of arthritis by others, consisted of gonococcus complement fixation and hemolytic streptococcus agglutination and precipitation tests, and antistreptolysin and antifibrinolysin determinations.

The patients studied were those comprising the groups already described in the preceding paper.<sup>1</sup> However, since certain of the tests were added during the course of the investigation, not all were performed on every patient. The subdivisions of arthritis were the same as used previously, as were the criteria for placing patients in the various groups.<sup>1</sup> In this regard it should be emphasized that the tests under investigation were never used in classifying cases. The necessity of adhering strictly to this rule is obvious since the purpose of the study was to evaluate the tests as aids in differentiation. In the case of gonococcus complement fixation, however, a positive reaction in a patient whose diagnosis was uncertain sometimes led to chemical or bacteriologic findings which had previously been missed and which allowed the case to be correctly classified. As in the study of blood cultures all doubtful cases were placed in the unclassified group.

The methods and results will be described separately for each test.

#### GNOCOCCUS COMPLEMENT FIXATION TESTS

Although the gonococcus complement fixation reaction has been known and used in the diagnosis of gonococcal infections for many years conflicting reports of its value are still seen. The belief that positive reactions are significant but negative ones of no value has arisen from the experience of urologists that known gonococcal urethritis may exist in the face of negative complement fixation. As noted by Price,<sup>2</sup> however, it has been shown that, while a large percentage of patients with early, localized anterior urethritis show negative reactions, the more chronic and more systemic infections tend to give positive fixation. Since joint involvement indicates that the disease has become systemic, it would be expected

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that cases of gonococcal arthritis would show positive complement fixation, and this has been borne out by a number of studies.<sup>3, 4, 5</sup>

*Method.*—The technique for performing the tests was that described by Park and Williams.<sup>6</sup> Standardized reagents were kindly furnished by Dr. Annis Thompson of the Bureau of Laboratories of the City of New York. The gonococcus antigen was prepared according to the method of McNeal.<sup>7</sup> Each test was controlled with known positive and negative serums.

*Results.*—The number of patients and the results obtained are recorded in Table I.

TABLE I

GNOROCOCBUS COMPLEMENT FIXATION SHOWN BY SERUMS OF ARTHRITIC PATIENTS AND CONTROLS

	NUMBER OF SERUMS	NUMBER OF PA- TIENTS	PA- TIENTS POSI- TIVE	PATIENTS SHOWING FOLLOWING REACTIONS				
				±	+	++	+++	++++
				PER CENT	PER CENT	PER CENT	PER CENT	PER CENT
Normal controls	36	35	0					
Miscellaneous diseases	74	69	6		1	1		4*
Active rheumatic carditis and chorea	34	23	5				5	
Rheumatic fever with polyarthritis	113	70	7		6	1		
Atrophic (rheumatoid) arthritis	65	39	3	3				
Gonococcal arthritis	88	44	98	7	2	36	33	26
Nonspecific, low grade pyogenic arthritis	41	30	0					
Osteoarthritis	58	48	2	2				
Miscellaneous arthritis (gout, spondylitis, tuberculous, etc.)	55	36	3*			3*		
Unclassified arthritis					5	7		
Unclassified infectious arthritis	71	44	12					
Wholly unclassified arthritis	42	30	0					
Totals	677	468						

Percentages are shown as nearest whole numbers.

\*Meningococcus meningitis.

The percentage of patients with gonococcal arthritis in this series whose serums fixed complement (98 per cent) is higher than that reported by others. The reason for this is not clear but perhaps a partial explanation lies in the fact that tests were repeated at intervals, if negative at the first bleeding. In several instances, for example, positive reactions were not obtained until the third bleedings, without which these cases would have been recorded as negative.

It will be noted that a number of patients with positive gonococcus fixation reactions were listed in the unclassified groups. Probably several of these had gonococcal arthritis but as they did not fulfill the requirements set for such a diagnosis (see paper I, page 457), they were listed as shown. That such positive complement fixation does not necessarily give the diagnosis of the joint disease is obvious, as illustrated by the patients with unquestionable rheumatic fever who happened to have coincidental gonococcal infections. The tests were, however, of great value, and it is felt, not only that positive results are important in establishing the diagnosis of gonococcal arthritis in a patient with joint disease, but also that repeatedly negative reactions are highly significant in excluding it.

## HEMOLYTIC STREPTOCOCCUS AGGLUTINATION TESTS

Interest in hemolytic streptococci as the possible cause of rheumatoid arthritis received great impetus from the success of Cecil, Nicholls, and Stainsby<sup>8</sup> in isolating these microorganisms from blood cultures of patients with that disease. Subsequently the same authors<sup>9, 10</sup> showed that serums of atrophic (rheumatoid) arthritic patients agglutinated their "typical strains" of hemolytic streptococci in high titers. In the same year, however, Dawson, Olmstead and Boots<sup>11</sup> demonstrated that such agglutinability is not limited to the "typical strains" of Cecil, Nicholls and Stainsby but is common to hemolytic streptococci isolated from patients with a variety of diseases. Thus although the results no longer supported the theory that a specific kind of hemolytic streptococcus was the cause of atrophic (rheumatoid) arthritis, the test was confirmed as of diagnostic importance in distinguishing this disease from other forms of arthritis. This has been confirmed in general, by the studies of Gray and Gowen,<sup>12</sup> Ashworth,<sup>13</sup> Keefer, Myers and Oppel,<sup>14</sup> Cox and Hill,<sup>1</sup> Wainwright,<sup>15</sup> and Blair and Hallman.<sup>17</sup> The results of Clawson and his coworkers<sup>18</sup> do not bear on the question since the strains used by them for agglutination apparently were not hemolytic.

*Method*—The method followed and the criteria used in reading the tests were those described by Dawson, Olmstead and Boots<sup>11</sup> whose article may be consulted by those wishing the details of technique. Following is a summary of the method. Twenty hour broth cultures of hemolytic streptococci in quantities of 0.5 cc were mixed with equal amounts of the various serum dilutions. Tests were read after incubation two hours in the water bath at 56° C and refrigeration overnight. The final dilutions were 1 to 20 through 1 to 640, since these six proved sufficient, higher dilutions were only rarely used. In the early part of the work, strain AB 13, kindly provided by Dr. Stainsby, was used, but was abandoned because, in our hands, it not infrequently agglutinated spontaneously in control tests with normal serums. After trial of a number of strains, all of which were more or less satisfactory, the well known hemolytic streptococcus, NY 5, originally isolated from the throat of a patient with scarlet fever, was selected for routine use. Occasionally, stock broth cultures kept in the ice box for long periods tended to give rise to granular suspensions, in which case it was found important to discard them and start afresh with new cultures from the frozen and dried stocks.<sup>19</sup>

*Results*—With this technique, results of great uniformity were obtained, as shown in Table II. The finding of others that hemolytic streptococci are agglutinated by serums of patients with atrophic (rheumatoid) arthritis is strikingly confirmed, indeed, the percentage of such patients showing agglutination in this series is higher than that in most other reports. This is perhaps due to the fact that in many clinics the name atrophic (rheumatoid) arthritis is given to cases of joint disease of the type here referred to as nonspecific low grade pyogenic arthritis and these patients in the present study gave uniformly negative agglutination. Whether this supports the clinical impression of the authors that these two groups of cases should be distinguished cannot, of course, be stated until the significance of hemolytic streptococcus agglutination in atrophic

study. It should be mentioned, too, that serums of four patients with bacterial endocarditis due to green streptococci (not included in the table) gave strongly positive precipitation with the crude C extract of hemolytic streptococci, as was noted previously by Seegal, Heidelberg, Jost and Lytle.<sup>25</sup>

TABLE III

PRECIPITIN REACTIONS BETWEEN CRUDE "C" EXTRACT OF HEMOLYTIC STREPTOCOCCUS (GROUP A) AND SERUMS OF ARTHRITIC PATIENTS AND CONTROLS

	NUMBER OF SERUMS	NUMBER OF PA- TIENTS	PA- TIENTS POSI- TIVE	PATIENTS SHOWING FOLLOWING REACTIONS			
				+	++	+++	++++
			PER CENT	PER CENT	PER CENT	PER CENT	PER CENT
Normal controls	22	21	24	14	5	5	
Hemolytic streptococcal diseases	14	8	62	12	12	12	25
Active rheumatic carditis and chorea	14	9	55	33	11		11
Rheumatic fever with polyarthritis	69	39	56	23	2	8	23
Atrophic (rheumatoid) arthritis, disease active	36	22	77	36	18		23
Gonococcal arthritis	32	19	32	32			
Nonspecific, low-grade pyogenic arthritis	17	13	31		23		8
Osteoarthritis	35	30	18	7	7	4	
Miscellaneous arthritis (gout, spondylitis, tuberculous, etc.)	19	16	12	12			
Unclassified arthritis							
Unclassified infectious arthritis	36	26	46	23	9	9	5
Wholly unclassified arthritis	26	20	58	33	8	8	8
Total	338	223					

Percentages are shown as nearest whole numbers.

#### ANTISTREPTOLYSIN DETERMINATIONS

Todd<sup>26</sup> in 1931 showed that streptococcal hemolysin is antigenic when suitably prepared, and that it gives rise to a species-specific antibody. Later he<sup>27</sup> showed the presence of this antibody (antistreptolysin) to be present only following hemolytic streptococcal infection; and both he<sup>28</sup> and Coburn and Pauli<sup>29</sup> reported high antistreptolysin titers to be constant findings in patients with active rheumatic fever. Myers and Keefer<sup>30</sup> found the antistreptolysin titer generally high but not constantly so in known hemolytic streptococcal diseases and rheumatic fever, but within normal limits in a few patients with atrophic (rheumatoid), gonococcal and hypertrophic (osteo-) arthritis. Wilson, Wheeler and Leask,<sup>31</sup> reporting their experience with antistreptolysin determinations in rheumatic children, concluded that a rise in titer is not a necessary accompaniment in such patients. Recently Blair and Hallman<sup>17</sup> obtained results similar to those of Myers and Keefer except that antistreptolysin titers definitely above normal were found in about one-third of their cases of atrophic (rheumatoid) arthritis.

*Method.*—The technique used was that of Hodge and Swift,<sup>6</sup> to whose article<sup>11</sup> the reader is referred for details. With this method, remarkably constant results were obtained with different lots of streptolysin throughout the study. Only pa-

\*Serum and streptolysin of known titers were kindly provided by Dr. Homer F. Swift and Dr. B. E. Hodge for the determination of standards for this study.

TABLE IV  
ANTISTREPTOLYSIN TITERS SHOWN BY SERUMS OF ALTHRITIC PATIENTS AND CONTROLS

	NUMBER OF SERUMS	NUMBER OF PATIENTS	PATIENTS SHOWING UNITS ANTISTREPTOLYSIN												
			<25	25	50	100	150	200	250	300	350	400	450	500	>500
Normal controls	15	37	% 11	% 40	% 13	% 23	% 7	% 7	% 7	% 16	% 5	% 13	% 0	% 0	% 10
Hemolytic streptococcal diseases	57	30													
Active rheumatic enditis and chorea	22	14													
Rheumatic fever with polyarthritia	84	51													
Atrophic (rheumatoid) arthritis	51	26	6	47	18	18	6	3		8	3				
Gonococcal arthritis	63	38	5	29	40	9	9								
Non-specific, low grade pyogenic ar- thritis	70	21	15	29	16	10									
Osteoarthritis	37	16	18	41	4	7									
Miscellaneous arthritis (gout, spondylitis tuberculous, etc.)	70	25	17	28	25	22									
Unclassified arthritis															
Unclassified infectious arthritis	99	40	5	17	25	25	13	7	2	7				0	
Wholly unclassified arthritis	11	11	1	11	18	22	9		4						
Total	548	19													

Percentages are shown as nearest whole numbers

\*Arthritis occurring during course of meningococcal meningitis

†Blood culture positive for hemolytic streptococcus in one of these two patients

5\*



tients who had test bleedings over a period of at least one month after the onset of illness were included in the analysis, the average in the arthritic groups being two and one-half months.

*Results.*—As shown in Table IV, all but two of the normal individuals gave readings of 50 units of antistreptolysin or less; however, it seemed best for the present to consider only titers above 150 units abnormal. Examination of the table shows that the readings above this figure were limited almost entirely to the patients with known hemolytic streptococcal diseases and active rheumatic fever without and with polyarthritis. The percentages of patients in these three groups with titers in the abnormal range were 43 per cent, 56 per cent, and 65 per cent, respectively. The percentage in the group of known hemolytic streptococcal cases (most of which were scarlet fever\*) was lower than had been expected. Probably it would have been higher had the patients been followed longer than the one-month period usual for this control group.

Only two patients with atrophic (rheumatoid) arthritis gave high titers. Of the two cases of gonococcal arthritis with antistreptolysin titers of 450 units, one was easily explained since the patient had a mixed gonococcal and hemolytic streptococcal septicemia; no explanation was apparent, however, for the high reading in the second case. Likewise, no reason could be found for the titer of over 500 units in a patient whose arthritis appeared during the course of meningococcal bacteremia. An interesting question naturally arises as to whether the eight patients with unclassified arthritis who showed readings in the abnormal range actually had atypical rheumatic fever.

It is clear that antistreptolysin titers were high in the majority of patients with rheumatic fever in this series, and it is probable that the percentage would have been even higher had the patients been followed still longer. On the other hand, the titer did not rise above 25 units in one unquestionable case followed more than two months.

#### ANTIFIBRINOLYSIN DETERMINATIONS

In 1933 Tillett and Garner<sup>33</sup> reported studies on the ability of hemolytic streptococci to liquefy normal human fibrin clot, and the following year<sup>34</sup> described the capacity of plasma clots from the blood of patients convalescent from acute hemolytic streptococcal infections to resist this fibrinolytic action. Hadfield, Magee and Perry<sup>35</sup> used the procedure in studying rheumatic children and found that in 48 per cent of 21 patients with active disease the clots were resistant to fibrinolysis whereas in all of 23 quiescent and 26 normal children the clots were susceptible. Myers, Keefer and Holmes<sup>36</sup> applied the test in a number of diseases and found the clots of practically all patients with known hemolytic streptococcal diseases and rheumatic fever more or less resistant while those of normal individuals were less resistant or susceptible. Their results in atrophic (rheumatoid) and gonococcal arthritis were similar to the normal except that occasional clots showed high resistance.

\*The serums from patients with scarlet fever were obtained from the Willard Parker Hospital, New York City.

*Method*—The technique of Tillett, Edwards and Garner<sup>34</sup> was followed exactly. Their classification of the degree of susceptibility of the clot was employed

Dissolution in less than 30 minutes	Highly susceptible
Dissolution in 30 to 60 minutes	Susceptible
Dissolution in 1 to 8 hours	Definite resistance
Dissolution in 8 to 24 hours	Marked resistance
No dissolution in 24 hours	Maximum resistance

Only patients whose test bleedings covered a period of at least one month after the onset of disease were included

TABLE V  
ANTIFIBRINOLYTIC ACTIVITY OF SERUMS OF ARTHRITIC PATIENTS AND CONTROLS

	NUMBER OF SERUMS	NUMBER OF PA- TIENTS	PER CENT PATIENTS WHOSE CLOTS DIS- SOLVED IN TIME SHOWN				
			< 1/4 HR.	1/4-1 HR.	1-8 HR.	8-24 HR.	> 24 HR.
			PER CENT	PER CENT	PER CENT	PER CENT	PER CENT
Normal controls	17	17	24	12	59		6
Hemolytic streptococcal disease	10	3					100
Active rheumatic carditis and chorea	10	6			66		33
Rheumatic fever with polyarthri- tis	43	29	10	3	14	14	59
Atrophic (rheumatoid) arthritis	11	11	18	27	36		18
Gonococcal arthritis	10	10	50	10	10		20
Nonspecific, low grade pyogenic arthritis	11	8	50		38		12
Osteoarthritis	13	13	54	8	38		
Miscellaneous arthritis (gout, spondylitis, tuberculous, etc)	13	11	36		27	9	27
Unclassified arthritis							
Unclassified infectious arthritis	25	19	27		27		46
Wholly unclassified arthritis	15	15	47		40	7	7
Total	176	146					

Percentages are shown as the nearest whole numbers

*Results*—The results summarized in Table V, were very similar to those obtained by Myers, Keefe and Holmes<sup>35</sup>. In general, they tended to parallel those of antistreptolysin determinations but were less clear cut in separating the various groups. The percentage of normal individuals in this small series whose clots did not liquefy within one hour was more than twice that reported by Tillett, Edwards and Garner<sup>34</sup>. This is perhaps due to a greater incidence of unnoticed mild hemolytic streptococcal infections in New York than in Baltimore, where their observations were made.

#### DISCUSSION

In view of the etiologic significance of the gonococcus complement fixation reaction in the type of arthritis in which it is generally positive, a question naturally arises as to the possibility of a similar significance for the other tests here described in rheumatic fever and atrophic (rheumatoid) arthritis. The

\*Through the kindness of Dr. William S. Tillett a culture of hemolytic streptococcus CO<sub>2</sub> was obtained for use in these tests.

results of agglutination tend to implicate the hemolytic streptococcus in atrophic (rheumatoid) arthritis but are usually negative in rheumatic fever; the reverse, however, is true for the antistreptolysin and antifibrinolysin tests, while precipitation reactions with hemolytic streptococcal fractions tend to be positive in both diseases. Other than to point out that these apparently contradictory results are actually not immunologically inconsistent, this phase of the problem will not be discussed further here. Neither can the bearing of the tests on the problem of relationship between rheumatic fever and atrophic (rheumatoid) arthritis be discussed in this paper which is concerned chiefly with the possible value of these procedures as differential diagnostic aids.

In the case of gonococcus complement fixation, hemolytic streptococcus agglutination and antistreptolysin tests, the results in this series of cases were sufficiently selective of certain forms of arthritis to warrant their further use as diagnostic aids. Hemolytic streptococcus precipitin and antifibrinolysin reactions tended to give results paralleling those of agglutination and antistreptolysin determinations respectively, but were far less selective. In Table VI are shown

TABLE VI

SCHEMATIC REPRESENTATION OF TENDENCIES SHOWN BY GONOCOCCUS COMPLEMENT FIXATION, HEMOLYTIC STREPTOCOCCUS AGGLUTINATION AND ANTISTREPTOLYSIN TESTS IN VARIOUS TYPES OF ARTHRITIS

	GONOCOCCUS COMPLEMENT FIXATION	HEMOLYTIC STREPTOCOCCUS AGGLUTINATION	ANTI- STREPTOLYSIN
Rheumatic fever with or without polyarthritis	-	-	+
Atrophic (rheumatoid) arthritis	-	+	-
Gonococcal arthritis	+	-	-
Nonspecific, low-grade pyogenic arthritis	-	-	-
Osteoarthritis	-	-	-
Gout, tuberculous arthritis, scurvy, etc.	-	-	-

the patterns of results obtained with the three more uniform tests in the various groups of joint diseases included in this study. With the addition of the erythrocyte sedimentation test to distinguish between osteoarthritis and what is here referred to as nonspecific, low-grade pyogenic arthritis, a literal acceptance of the tendencies represented in Table VI might lead to the conclusion that the time is at hand when a differential diagnosis in joint disease may be made without looking at the patient merely by sending a specimen of blood to the laboratory. Obviously such is far from the case, for the exceptions to the results in almost every category are sufficient to raise some doubt as to the specificity of the tests in question. Furthermore, the cases of classified arthritis on which the results reported here were based were all characteristic examples of their respective types and easily classified by purely clinical means. Hence, it follows that the results with the various tests might be fairly definite in these cases in which they are not necessary for diagnosis, and yet be vague in the less clearly defined and early cases in which help is most needed.

It must be emphasized, too, that the total experience with most of these techniques is small thus far, and that objections may become apparent with their further use. Already, discrepancies have appeared in the results of various work-

ers Cox and Hill,<sup>1</sup> for example obtained positive hemolytic streptococcus agglutination in a number of patients with osteoarthritis, Wilson, Wheeler and Leask<sup>2</sup> apparently consider antistreptolysin determinations of little significance in rheumatic fever, and recently Blair and Hallman<sup>3</sup> reported abnormally high antistreptolysin titers in almost one third of their cases of atrophic (rheumatoid) arthritis. The lack of exact criteria for placing patients in the various arthritic groups adds to the difficulty of interpreting the results reported.

In spite of the obvious deficiencies of the tests at the present stage of their development, the authors believe that gonococcus complement fixation, hemolytic streptococcus agglutination and antistreptolysin determinations are distinctly useful in the study and differentiation of joint disease and that they might well be included as routine procedures in arthritis clinics.

This investigation is being continued with particular reference to clinically atypical, unclassified cases. It is hoped that by carefully following such patients over a period of years the correct diagnoses may become clear in enough instances to warrant a decision as to the significance of positive tests in cases not diagnosable clinically. It may thus be learned whether these methods are of more than confirmatory value and may suggest a correct diagnosis earlier than would be possible without them.

#### SUMMARY

As part of a wider study of methods which might prove useful in the differential diagnosis of joint diseases gonococcus complement fixation, hemolytic streptococcus agglutination and precipitation tests, and antistreptolysin and antifibrinolysin determinations were made on a series of patients with various types of arthritis. The results obtained with each have been discussed. The precipitation and antifibrinolysin tests in this study gave less selective results than the others. Gonococcus complement fixation reactions were positive in 98 per cent of 44 patients with gonococcal arthritis. The serums of 86 per cent of 36 patients with active atrophic (rheumatoid) arthritis agglutinated a hemolytic streptococcus. Antistreptolysin titers above normal were obtained in at least 66 per cent of 51 patients with rheumatic polyarthritis. These three tests were considered distinctly useful.

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## DISCUSSION

DR. RUSSELL L. CECIL, New York, N. Y.—I am much interested in Dr. McEwen's report, particularly that part which has a bearing on studies which Nicholls, Stainsby and I published some years ago. Dr. McEwen's figures differ from ours in several respects. The percentage of positive blood cultures in the arthritic patients was lower and the percentage of positive cultures in the controls was higher than in our series.

Most of Dr. McEwen's strains were classified as viridans. In our study, we were at first dubious about the classification of these peculiar streptococci. In our preliminary report we called them viridans. After they had been kept under cultivation for some time, a good many of them took on hemolytic qualities. We should not allow ourselves to be confused by the terminologies of different workers, for many streptococci undergo mutations under certain conditions. The higher percentage of positive blood cultures in Dr. McEwen's control groups throws some light on this problem. In our original study I believe we made the mistake of not having a wider variety of controls. Most of them were either normal, healthy controls or patients with hypertrophic arthritis. In our normal healthy controls we had no positive cultures; in our other group we had a small percentage of positives. If we had run more controls on patients with other forms of infection, especially acute respiratory infections, we might have avoided that error.

I was particularly interested in Dr. McEwen's high percentage of positive agglutinations. I think the percentage of positive agglutinations to hemolytic streptococci with arthritic serum will depend on how the cases are selected. You will find both high positive cultures and high agglutinations if you are careful in selection of cases. If you are not careful the percentage will be low. It is interesting that in spite of the large group of controls that showed streptococci in the blood, the group of cases of atrophic arthritis is the only one that showed a high percentage of agglutination reactions. The significance of streptococci in the blood of arthritic patients is, of course, influenced somewhat by the presence of streptococci in controls. The next important problem in this field is to determine the origin of these streptococci. Dr. McEwen did not show, but I think he has evidence, as we had, that the streptococci are actually in the blood stream and not contaminations from the skin or air. We must find out how they get there.

DR. M. H. DAWSON, New York, N. Y.—May I say a few words regarding the reliability of the agglutination reaction with hemolytic streptococci in atrophic (rheumatoid) arthritis. Prior to July of last year we had tested the serums from 503 cases of atrophic (rheumatoid) arthritis and from 571 control cases and during the past year we have tested

several hundred more cases. In our experience the reaction is a most significant one and only occurs in atrophic (rheumatoid) arthritis or in severe hemolytic streptococcal infections. We have never found a positive reaction in any case of hypertrophic arthritis. We have found the test to be as valuable as the gonococcal complement fixation test in gonorrheal arthritis.

One would not expect to find the antistreptolysin and antifibrinolysin tests positive in a chronic disease such as atrophic (rheumatoid) arthritis. These tests are indices of recent acute hemolytic streptococcal infection. However, if one confines oneself to cases of early acute atrophic (rheumatoid) arthritis one is impressed with the percentage of cases showing an increase in the antistreptolysin content of the serum.

DR J. A. KEY, St. Louis, Mo.—I am surprised that no staphylococci grew in these cultures, even as contaminations. In doing synovectomies, I am still able to excise a piece of the synovial membrane, immediately put it in broth, and grow staphylococci therefrom in about one case in three. I have never gotten streptococci from a chronic arthritic joint. I do not know why they will not grow for me, but they do not.

I wonder whether or not cases that give a positive complement fixation test are really of gonorrheal arthritis. Would other gonorrheal infections present in the same individual account for positive tests? I wonder if a coincident (nongonorrheal) arthritis would not be called gonorrheal arthritis because a case gave a positive fixation test.

DR JOHN W. GRAY, Newark, N. J.—Dr. McEwen's bacteriologic and serologic findings in atrophic (rheumatoid) arthritis are similar to ours. We have felt that our high percentage of blood cultures positive for streptococci was of significance and that positive agglutinations strengthened this assumption. In a small percentage of our cases the blood cultures showed staphylococci and although further studies may show their relationship to the disease, we should, until that time, consider them contaminants.

DR MCEWEN (closing).—We had a fair percentage of contaminants, and we occasionally noted staphylococci in cultures. We considered them contaminants and did not therefore include them in the table.

I agree with Dr. Dawson that antistreptolysin titers are apt to be high in patients with early atrophic (rheumatoid) arthritis, while they are seldom high in more advanced cases.

The possibility that positive gonococcus complement fixation tests might lead to a false diagnosis in some patients, was borne in mind. We therefore set strict criteria for the diagnosis of gonococcal arthritis. As I said in the beginning, the tests we were evaluating were not used in placing patients in the various groups. The gonococcus complement fixation test was an exception to this only in the sense that a positive result in a patient in whom gonococcal arthritis had not been suspected led us to examine him for further clinical evidence to establish that diagnosis.

Concerning Dr. Dawson's statement on the specificity of hemolytic streptococcus agglutination in atrophic (rheumatoid) arthritis, I would point out that occasional patients with rheumatic fever gave a positive agglutination reaction. This was only rarely encountered.

# PROTEIN STUDIES IN ATROPHIC (RHEUMATOID) AND HYPERTROPHIC ARTHRITIS

JOHN STAIGE DAVIS, JR., M.D., NEW YORK, N. Y.

SINCE the reawakening of interest in the sedimentation rate by Robin Fareus<sup>1</sup> in 1918, there has been an immense amount of clinical and laboratory investigation concerning the chemical changes in the blood which are responsible for this phenomenon.

Boots, Dawson and Sia<sup>2</sup> have called attention to a difference in sedimentation rate in atrophic arthritis as opposed to that in hypertrophic arthritis. They stated that this was further evidence to show that these were two different and distinct diseases.

Snapper and others<sup>3</sup> showed that the power of the red cells to settle at various rates in different diseases was dependent on three factors: i.e., cell volumes, plasma fibrinogen, and plasma globulin. If these three constituents be known, the sedimentation rate can be calculated with a fair degree of accuracy.

It was in an effort to determine just how these changes occur in the various types of arthritis that this work was undertaken. Also, it was hoped that by a study of the different globulin fractions of the plasma, some tangible means of determining the effect of various forms of treatment might be obtained. Rubinstein and Fischer,<sup>4</sup> Hurwitz and Meyer,<sup>5</sup> and others<sup>6</sup> have shown that a rise in globulin, especially in the euglobulin fraction, could be obtained by the administration of typhoid vaccine and that such a rise occurs in bacterial infections. It was thought that a similar rise might be obtained from the administration of either hemolytic streptococcus vaccine or different types of antogenous vaccines in the treatment of arthritis. If such a rise would result then one would expect an increase in the sedimentation rate as desensitization took place, rather than a decrease, as is most commonly seen. The effects of several forms of treatment on the blood protein are now being studied and will be reported at a later date.

This paper will deal only with those changes which occur at various stages of the disease, arthritis.

## MATERIAL AND METHODS

The total plasma protein, albumin, globulin, fibrinogen, globulin fractions pseudo I and II, and euglobulin and sedimentation rate have been determined on numerous occasions on ninety subjects. The cases are divided as follows:

Normals	9
Serious atrophic (rheumatoid) arthritis	15
Acute rheumatic fever	1
Arrested cases of atrophic (rheumatoid) arthritis	6
Mild cases of atrophic (rheumatoid) arthritis	20
Cases ill with other diseases than arthritis	6
Myositis	5
Cases of hypertrophic arthritis	11
	<hr/> 76

The protein contents have been determined on 220 samples of blood. This paper is based on the findings obtained from 76 of the 90 subjects concerning whom the diagnosis was definite. For example, those who were unfortunate enough to have both types of arthritis or those with gonorrheal arthritis are not included in this report.

The chemical analyses were done by Dr. Chandler in the Department of Physiology at the Cornell Medical School. The method of Howe has been used. The sedimentation rates were done according to modification of Westergren method, no correction for cell volumes has been made. The cases have been obtained from St. Luke's Hospital, the New York Post Graduate Hospital and from private practice.

## RESULTS

In Table I are given the results obtained from an analysis of the blood of nine normals. These figures compare favorably with the average normal con-

TABLE I

NORMAL

NAME	DATE	TOTAL PROT	W.B	GLOB	RATIO	FIBRIN	PSEUDO I	PSEUDO II	UL GLOB	SED RATE
W B E	9/25/34	6.36	1.32	2.04	2.12	0.75	1.0	0.40	0.25	10
F L	6/27/34	6.38	4.40	1.92	2.22	0.24	1.71		0.27	
M L	6/27/34	7.35	5.22	2.13	2.35	0.33	1.13	0.14	0.54	
G P	10/30/34	6.87	4.43	2.44	1.91	0.43		0.54		10
P H	4/16/35	6.84	4.67	2.17	2.15	0.71	1.11	0.33	0.40	13
J S D	3/12/34	6.40	4.15	2.22	1.88	0.20	1.32	0.40	0.20	
B M	11/20/34	7.23	1.02	2.40	2.01	0.50	1.26	0.41	0.23	15
C C	6/23/34	7.25	2.05	2.17	2.28	0.26	1.39		0.42	
E S	4/30/35	7.32	1.99	2.31	2.14	0.20	1.15	0.52	0.20	
Average		6.91	1.70	2.21	2.12	0.45	1.17	0.40	0.3	12

TABLE II

SEVERE ATROPHIC (RHEUMATOID) ARTHRITIS

NAME	DATE	TOTAL PROT	W.B	GLOB	RATIO	FIBRIN	PSEUDO I	PSEUDO II	UL GLOB	SED RATE
M M	2/20/34	7.54	2.56	2.98	0.90	0.65	1.59	0.66	0.75	
L M	2/27/34	7.22	3.59	2.6	0.99	0.81	1.58	0.71	0.50	125
T F	6/26/34	8.15	3.66	4.49	0.82	0.77	2.01	0.68	1.00	45
B K	5/ 8/35	5.70	2.32	3.38	0.69	0.38	1.70	0.52	0.57	
O F	7/ 2/34	7.75	1.59	2.86	1.01	0.57	2.49		0.80	97
C K	3/19/34	7.1	2.21	4.14	0.77	0.65	2.06	0.65	0.78	
E L	10/21/34	7.98	3.92	4.06	0.97	0.51	2.53		0.78	
O O	3/23/34	6.01	2.32	2.77	0.71	0.36	1.15	0.62	0.71	
L H	2/20/34	8.25	4.08	4.27	0.96	0.45	1.29	0.58	0.85	47
L	7/12/34	6.77	2.01	3.72	0.81	0.61	1.61	0.50	0.70	120
H L	1/22/35	7.84	2.97	2.87	1.02	0.60	1.81	0.69	0.73	56
A M	4/27/34	6.61	1.4	2.8	1.07	0.62	1.22	0.55	0.70	
T J	6/12/34	7.74	4.1	4.23	0.80	0.54	1.1	0.90	0.97	
M G	2/20/34	7.1	7.1	1.1	1.01	0.9	1.81	0.68	0.68	101
M C	9/11/34	8.1	8.1	1.2	0.91	0.19	2.02	0.70	0.91	65 Rb fever
F J	10/2 / 34	7.22	6	0	0.98	0.59	2.11		0.42	
Average		7.6	4.9	8.5	0.90	0.62	1.81	0.68	0.74	85



TABLE III  
HYPERTROPHIC ARTHRITIS

NAME	DATE	TOTAL PROT.	ALB.	GLOB.	RATIO	FIBRIN.	PSEUDO I	PSEUDO II	EUGLOB.	SED. RATE
E. O.	5/ 1/34	6.75	4.25	2.51	1.69	0.20	1.39	0.57	0.44	5
E. S.	3/ 2/34	6.29	4.05	2.24	1.80	0.23	1.00	0.58	0.43	
J. H.	7/ 3/34	7.03	4.42	2.61	1.69		1.39	0.41	0.61	
D. K.	12/11/34	8.02	5.04	2.91	1.73	0.27	1.76	0.45	0.43	35
M. R.	4/17/34	6.90	4.34	2.56	1.70	0.32	1.28	0.54	0.42	45
M. Z.	3/13/34	6.57	4.06	2.51	1.62	0.13	2.27		0.11	
N. D.	4/10/34	6.85	4.29	2.56	1.68	0.42	1.25	0.59	0.30	50
C. H.	10/23/34	7.17	4.61	2.56	1.85	0.45	1.86		0.25	20
H. M.	9/18/34	7.58	4.63	2.95	1.59	0.36	1.60	0.45	0.54	10
I. S.	6/ 5/34	6.54	4.08	2.46	1.66	0.56	1.08	0.56	0.26	15
S. B.	3/ 6/34	7.29	4.26	3.03	1.40	0.46	1.44	0.62	0.43	31
M. S.	4/16/35	6.60	4.34	2.26	1.92	0.32	1.19	0.54	0.21	10
A. S.	1/ 8/35	7.38	4.90	2.48	1.92	0.28	1.26	0.48	0.46	25
K. Z.	3/20/34	7.25	4.54	2.71	1.67	0.26	1.14	0.69	0.62	85
Average		7.02	4.42	2.60	1.71	0.33	1.32	0.54	0.39	30

TABLE IV

RHEUMATOID ARTHRITIS (CHRONIC INFECTIOUS ARTHRITIS)	HYPERTROPHIC ARTHRITIS (OSTEOARTHRITIS)
Atrophic arthritis	Degenerative arthritis
Proliferative arthritis	
Primary progressive polyarthritis	
HISTORY	HISTORY
Sometimes present in family	Rarely present in family
Rheumatic fever at times	No rheumatic fever
Colds, sore throats, sinusitis	Unimportant
Indigestion	Unimportant
Underweight, undernourished	Overweight
Occasionally slight fever	No fever
Focus often present	Focus rarely present
<i>Joints first involved</i>	<i>Joints first involved</i>
Smaller joints of hands, elbows, knees, shoulders, ankles	Weight-bearing joints { knees hips spine
	Distal phalangeal joints—so-called "Heberden's nodes"
<i>Type of development</i>	<i>Type of development</i>
Soft tissue with later destruction of cartilage and bone	Bony involvement with deposit of calcium
<i>X-ray findings</i>	<i>X-ray findings</i>
None early. Later, bone rarefaction, destruction, deformity and ankylosis.	Increased density and spurring
Muscular atrophy	No atrophy
Progress to deformity	Does not progress to deformity
Cardiac involvement in a small percentage	No cardiac involvement
<i>Extremities:</i> cold, often	<i>Extremities:</i> warm
Often present: { Hypertrichosis Psoriasis	Rarely present
Subcutaneous nodules in 20%	Never present
LABORATORY FINDINGS	LABORATORY FINDINGS
Often anemic	Rarely anemic
Slight leucocytosis, sometimes	No leucocytosis
Increased sedimentation rate	Normal sedimentation rate
<i>Streptococcus hemolyticus</i> agglutination sometimes positive	Never positive
Increased blood globulin	Normal globulin

tent as determined by Van Slyke and others. His average normal figures obtained were

	MAXIMUM	MINIMUM
Total protein	75	56
Albumin	49	34
Globulin and fibrinogen	29	22
Albumin/globulin ratio	2.0	1.4

The results as obtained in 15 cases of severe atrophic (rheumatoid) arthritis and one case of rheumatic fever are given in Table II, while those obtained in 14 cases of hypertrophic arthritis are shown in Table III. Atrophic (rheumatoid) arthritis was differentiated clinically from hypertrophic arthritis on the basis of Table IV.

In Table V are given the results obtained from the study of the blood of six arrested cases. By arrested is meant those cases of long duration in which activity has practically ceased.

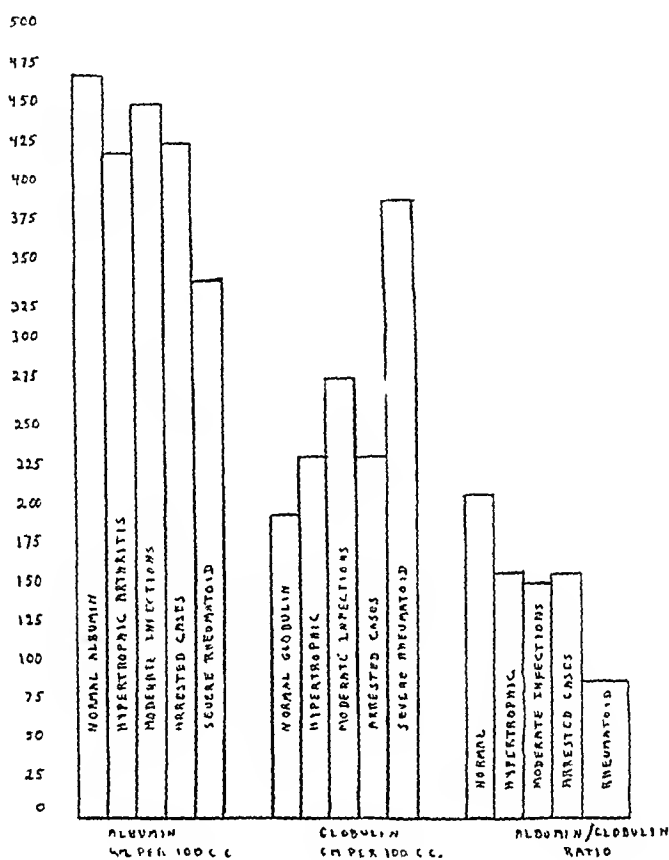
TABLE V  
ARRESTED CASES OF ATROPHIC (RHEUMATOID) ARTHRITIS

NAME	DATE	TOTAL PROT	ALD	GLOB	RATIO	FIBRIN	PSEUDO I	PSEUDO II	EUGLOB	SED RATE
E H	9/11/34	7.13	3.94	3.19	1.34	0.61	1.77	0.63	0.58	45
M C	4/ 2/34	6.71	3.35	2.86	1.35	0.62	1.37	0.54	0.33	
M P	10/ 2/34	6.87	4.54	2.33	1.95	0.32	0.68	0.56	0.47	30
A K	5/ 8/34	7.68	4.92	2.76	1.78	0.28	1.47	0.62	0.39	28
S	3/ 8/34	6.82	4.12	2.70	1.5	0.52	1.15	0.45	0.58	62
A S	5/ 8/34	7.41	4.43	2.98	1.48	0.38	1.57	0.58	0.45	50
F	9/25/34	7.01	4.07	2.94	1.38	0.45	1.34	0.65	0.50	

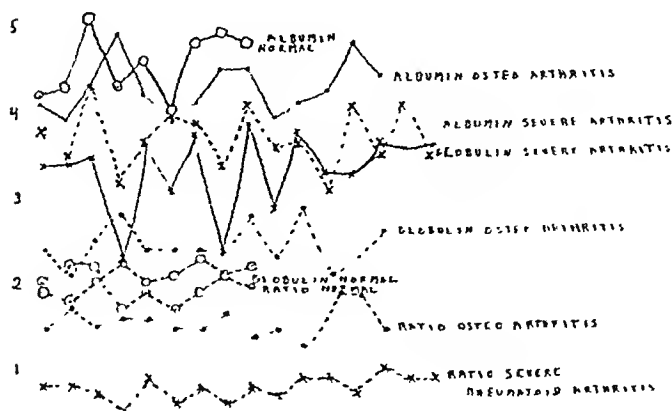
TABLE VI  
MODERATE ATROPHIC (RHEUMATOID) ARTHRITIS

NAME	DATE	TOTAL PROT	ALD	GLOB	RATIO	FIBRIN	PSEUDO I	PSEUDO II	EUGLOB	SED RATE
G K	4/10/34	7.89	4.76	3.13	1.52	0.40	1.50	0.65	0.55	80
T	4/ 7/34	6.91	4.32	2.59	1.66	0.50	1.38	0.50	0.21	
C	11/20/34	7.07	3.8	3.22	1.20	0.55	1.54	0.85	0.28	25
L I	9/18/34	7.57	4.43	3.14	1.41	0.59	1.34	0.71	0.50	42
F	11/ 3/34	7.62	4.27	3.35	1.27	0.64	1.63	0.51	0.58	
M	4/17/34	7.27	4.08	3.19	1.28	0.52	1.75	0.44	0.48	
H	9/25/34	7.08	4.53	2.55	1.78	0.45	1.46	0.38	0.26	20
C R	5/ 9/34	7.73	4.79	2.94	1.65	0.53	1.51	0.69	0.58	
E H	6/26/34	7.27	4.59	2.68	1.71	0.55	1.48	0.60	0.27	30
G P	6/12/34	7.25	3.62	3.43	1.11	0.47	1.58	0.66	0.72	
L K	6/19/34	7.57	4.40	3.17	1.39	0.29	1.68	0.62	0.58	45
F S	11/20/34	7.38	4.28	3.10	1.38	0.78	1.5	0.64	0.33	30
J B	10/23/34	8.05	4.91	3.14	1.56	0.66	2.06		0.22	35
G K	2/20/34	6.06	3.82	2.24	1.71	0.76	0.92	0.69	0.27	
J B	4/24/34	6.72	4.35	2.37	1.63	0.49	1.02	0.47	0.9	10
B S	3/13/34	7.41	4.28	3.13	1.37	0.11	1.82	0.65	0.32	
A N	5/29/34	7.03	3.86	3.17	1.21	0.61	1.17	0.51	0.59	15
H P	5/ 6/35	7.84	4.36	3.48	1.25	0.48	1.92	0.61	0.17	
A H	10/ 2/34	7.50	4.35	2.95	1.50	0.56	1.15	0.70	0.51	60
D C	5/14/35	7.42	4.62	2.80	1.65	0.52	1.39	0.62	0.47	
Average		7.32	4.33	2.98	1.47	0.51	1.46	0.61	0.45	35

The chemical changes which occur in the blood of 16 mild atrophic (rheumatoid) cases are shown in Table VI.



Graph 1.



Graph 2.

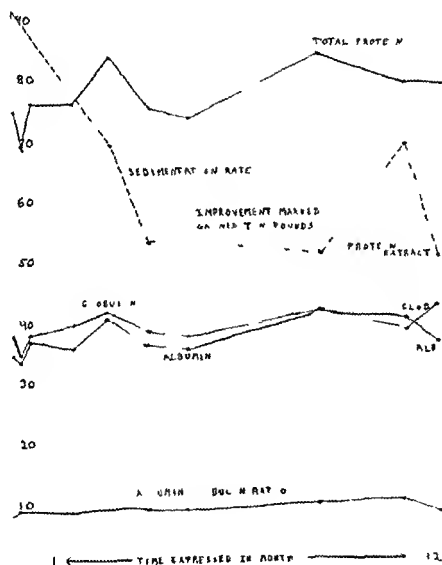
Graphs 1 and 2 are made from a summary of the albumin-globulin and albumin/globulin ratios given in Tables I, II, III, V, and VI.

In Graph 3 are shown the differences in the euglobulin fractions referred to above. It can be seen here that the greatest changes take place in the euglobulin fractions.

One patient, M M, has been followed over a period of one year. She has a very severe atrophic (rheumatoid) arthritis which fluctuates considerably from time to time. The changes in her blood protein fractions, as well as in her clinical course, are given in Table VII and Graph 4. The relationship between



Graph 3



Graph 4

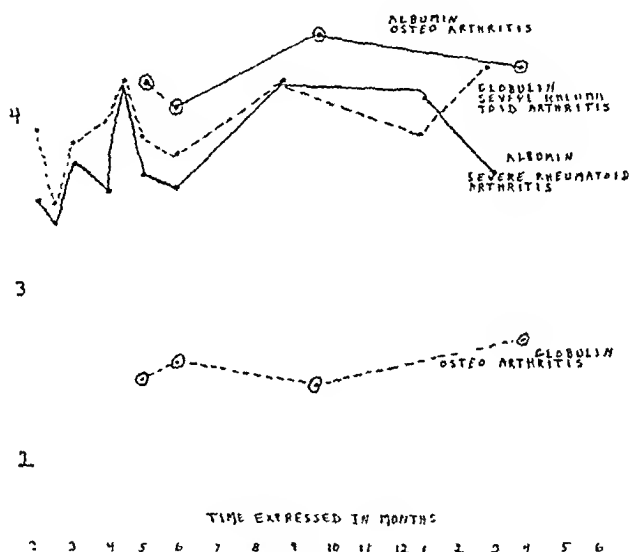
the plasma albumin and plasma globulin values of the blood of this patient M M and the patient E O, ill with hypertrophic arthritis, is shown in Graph 5.

One patient, J B, who made a complete recovery from a very acute and debilitating atrophic (rheumatoid) arthritis, was seen at weekly intervals during his illness and on three occasions his plasma proteins were determined

These findings have been recorded in Table VIII. It is of interest that shortly after his recovery his albumin/globulin ratio had risen above the normal range. This has been noticed in one other patient, who made a sudden and complete recovery. It seems to be, as it were, an over-compensation which will later return to a normal level.

Three patients, J. J., B. K., and M. L., had very severe atrophic (rheumatoid) arthritis, associated with considerable edema. The plasma albumin in all three instances was quite low, as can be seen from a study of Table II. It is suggested that this lowering of plasma protein may play some part in the production of the edema and that perhaps a diet high in protein is advisable. Such experiments are now being made.

In Table IX are given a series of findings from the blood of patient E. O., ill with hypertrophic arthritis. This patient has shown little clinical change.



Graph 5.

O. F., a patient ill with severe atrophic (rheumatoid) arthritis, improved following a transfusion of whole blood, in addition to various other forms of treatment, but has recently become worse. The findings in his case are given in Table X.

H. I., a patient who had been crippled with rheumatoid arthritis for six years and whose disease was considered to be arrested, was progressively improving until recently, when she became acutely ill with bronchitis. During the illness her arthritis again became active. The changes which took place in her plasma protein are shown in Table XI.

In Table XII are given the findings obtained in five cases of myositis. These figures fall within the normal range.

In order to have controls in other pathologic states, the plasma proteins of six patients ill with other diseases were determined.

TABLE VII

A PATIENT WITH SEVERE ATROPHIC (RHEUMATOID) ARTHRITIS WHO SHOWED NO CHANGE OVER A PERIOD OF ONE YEAR

NAME	DATE	TOTAL PROT	ALB	GLOB	RATIO	FIBRIN	PSEUDO I	PSEUDO II	EUGLOB	SED RATE	REMARKS
M M	2/20/34	7.54	3.56	3.98	0.90	0.68	1.89	0.66	0.70	92	Put on high protein diet. Extensive involvement
	2/27/34	6.93	3.42	3.51	0.90	0.45	1.89	0.62	0.54		No change
	3/6/34	7.65	3.79	3.86	0.98	0.55	1.95	0.60	0.76		Feels better
	4/3/34	7.65	3.64	4.01	0.91	0.91	1.91	0.62	0.66		Feels fairly well
	4/24/34	8.41	4.17	4.24	0.98	0.63	1.94	0.65	1.02	70	Has been taking protein for 1 month. Is improved
	5/15/34	7.61	3.70	3.91	0.95	0.48	1.67	0.68	1.08	55	About the same
	6/12/34	7.44	3.61	3.83	0.94	0.50	1.84	0.58	0.91	55	Gained 2 pounds. Not so well. Pulse rapid. Heart bothering.
	9/18/34	8.42	4.21	4.21	1.00	0.53	1.90	0.57	1.20	48	Gained 7 pounds. Feels pretty well
	1/8/35	8.11	4.16	3.95	1.00	0.39	1.66	0.80	1.10	70	Gained 2 pounds. Taking protein
	3/12/35	9.08	3.74	4.34	0.87	0.64	2.00	0.73	0.97	55	No protein in 6 weeks. Hands, knees, ankles swollen

TABLE VIII

A CASE OF MODERATE ATROPHIC (RHEUMATOID) ARTHRITIS WITH COMPLETE RECOVERY

NAME	DATE	TOTAL PROT	ALB	GLOB	RATIO	FIBRIN	PSEUDO I	PSEUDO II	EUGLOB	SED RATE
J B	10/23/34	8.05	4.91	3.14	1.56	0.86		2.06	0.22	35
	12/8/34	7.23	4.94	2.29	2.16	0.40	1.12	0.34	0.47	3
	3/19/35	6.94	4.97	1.97	2.52	0.24	0.85	0.61	0.27	

TABLE IX

CASE OF HYPERTROPHIC ARTHRITIS SHOWING NO CLINICAL CHANGE

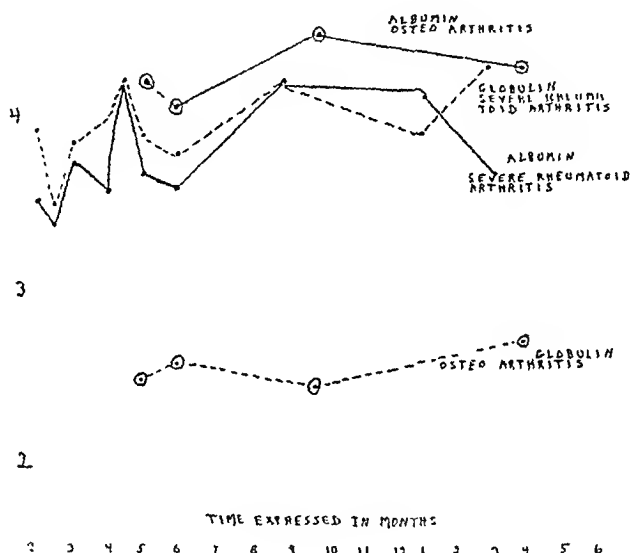
NAME	DATE	TOTAL PROT	ALB	GLOB	RATIO	FIBRIN	PSEUDO I	PSEUDO II	EUGLOB	SED RATE	REMARKS
E O	5/1/34	6.76	4.25	2.51	1.69	0.20	1.50	0.57	0.44	5	Pain in knees. Heberden's nodes
	6/19/34	6.77	4.12	2.61	1.57	0.40	1.36	0.49	0.36	20	Omnadin 1 amp weekly
	10/2/34	7.04	4.55	2.49	1.52	0.16	1.37	0.74	0.22		No change
	4/16/35	7.07	4.2	2.75	1.57	0.56	1.28	0.54	0.77	25	No change

From Table XIII it can be seen that changes occur in other diseases similar to those which occur in atrophic (rheumatoid) arthritis, but perhaps not quite so marked. It is also of interest that in the case of liver disease studied here, the fibrinogen rise is out of proportion to the other blood changes.

These findings have been recorded in Table VIII. It is of interest that shortly after his recovery his albumin/globulin ratio had risen above the normal range. This has been noticed in one other patient, who made a sudden and complete recovery. It seems to be, as it were, an over-compensation which will later return to a normal level.

Three patients, J. J., B. K., and M. L., had very severe atrophic (rheumatoid) arthritis, associated with considerable edema. The plasma albumin in all three instances was quite low, as can be seen from a study of Table II. It is suggested that this lowering of plasma protein may play some part in the production of the edema and that perhaps a diet high in protein is advisable. Such experiments are now being made.

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Graph 5.

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In Table XII are given the findings obtained in five cases of myositis. These figures fall within the normal range.

In order to have controls in other pathologic states, the plasma proteins of six patients ill with other diseases were determined.

suggested<sup>11</sup> that the rise in globulin is in a way an immunologic reaction and that the species of globulin varies with the disease.<sup>12</sup>

Epstein,<sup>13</sup> Van Slyke<sup>14</sup> and others have demonstrated that in certain renal diseases, edema is associated with a lowering of plasma albumin and sometimes with plasma globulin and that in such cases restriction of protein is dangerous. Peters and Van Slyke<sup>15</sup> state "that the deficit following either proteinuria or

TABLE XIII  
PLASMA PROTEIN FINDINGS IN DISEASES OTHER THAN ARTHRITIS

NAME	DATE	TOTAL PROT	ALB	GLOB	RATIO	MBIN	1ST UGOL I	1ST UGOL II	EUGLOB	SED RATE	REMARKS
E B	1/ 5/35	7.16	3.74	3.42	1.09	0.33	1.93	0.67	0.49	10	Central nervous system syphilis. Aneurysm.
T F	4/12/34	6.24	3.81	2.43	1.57	0.48	1.00	0.51	0.44	24	Paralysis agitans 7 years.
P R	5/ 3/34	5.65	4.01	1.64	2.44	0.21	0.99	0.25	0.18		Severe uricemia. Adrenalin administered, very restricted diet.
K C	4/22/35	7.23	4.26	2.97	1.47	0.70	1.15	0.70	0.39	40	Tumor of liver.
U M	4/ 9/35	6.26	3.41	2.85	1.26	0.45	1.12	0.72	0.56		Acute cholecystitis.
A D	1/10/35	7.49	4.49	3.00	1.50	0.27	1.45	0.73	0.55		Pernicious anemia.

TABLE XIV  
BLOOD PROTEIN CHANGES IN ACUTE ARTHRITIS OF SHORT DURATION

NAME	DATE	TOTAL PROT	ALB	GLOB	RATIO	MBIN	PSEUDO I	1ST UGOL II	EUGLOB
J D	3/12/34	6.40	4.15	2.22	1.58	0.30	1.32	0.40	0.20
	2/11/35	5.98	3.35	2.63	1.27	0.42	1.24	0.53	0.44

malnutrition falls, as a rule chiefly on the albumin fractions and is accompanied by a tendency to edema. The edema of degenerative Bright's disease appears certainly and edema of various malnutrition states probably to be due to albumin deficit in blood plasma."

In the light of this knowledge our findings would suggest that atrophic (rheumatoid) arthritis is an infectious disease, possibly associated with malnutrition, while hypertrophic arthritis is not, for in atrophic (rheumatoid) arthritis is found a lowered plasma albumin and an increase in plasma globulin. The major increase takes place in the euglobulin fraction. There is little or no change in the plasma protein in hypertrophic arthritis.

Furthermore, in certain cases of atrophic (rheumatoid) arthritis, particularly those associated with easily demonstrable edema it seems unwise to restrict protein in the diet. It has been said that protein will remove fluid from the tissue, because it takes fluid to excrete the protein. Such is not the case with carbohydrate. Pemberton<sup>16</sup> has demonstrated water retention in individuals on high carbohydrate diets.



As in other infectious diseases, the plasma protein in the patient ill with atrophic (rheumatoid) arthritis tends to return to a normal level as the patient recovers.

There is also an increase in fibrinogen in the blood of the atrophic (rheumatoid) arthritic patient. Usually when the fibrinogen is highest the globulin rises the least.

The sedimentation rate is a rough way of determining whether there is a rise in plasma globulin, or fibrinogen, or both, provided there is no marked change in cell volumes.

### CONCLUSIONS

1. Globulin fraction tends to rise in atrophic (rheumatoid) arthritis.
2. The greatest change takes place in the euglobulin fraction.
3. Albumin tends to fall in atrophic (rheumatoid) arthritis.
4. There is little, if any, change in the protein fraction of those ill with hypertrophic arthritis.
5. Further evidence is presented to show that atrophic (rheumatoid) arthritis is an infectious disease, while hypertrophic arthritis is not.
6. The sedimentation rate is a simple way of determining in a rough manner whether there has been a change in the globulin or fibrinogen, or both, providing a correction is made for the cell volume.
7. Theoretically, a diminished sedimentation rate is not always an accurate index of improvement as accomplished by vaccine, for a rise in globulin has been produced by certain vaccines and this per se might increase the sedimentation rate. This has not been our experience in using hemolytic streptococcus vaccine.
8. Fibrinogen content usually rises in atrophic (rheumatoid) arthritis but not in proportion to the globulin rise.
9. Restriction of protein in the diet of the atrophic (rheumatoid) arthritic patient might at times be dangerous.
10. Vitamin deficiency and malnutrition may play a part in the protein changes in atrophic (rheumatoid) arthritis.

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## DISCUSSION

DR RALPH PEMBERTON PHILADELPHIA PA—I am chiefly interested in Dr Davis' report, for two reasons, because of the implications it bears toward the nutritional aspects of chronic arthritis, and because Dr Scull working in our laboratory, met with somewhat comparable figures about a year ago in a series of fourteen cases. The figures we obtained are not so striking as those Dr Davis reports but they are not out of line with his and suggest that it may be worth while for us to take the matter up again and pursue it further.

Dr Davis' observations are also in line with the suspicion raised in the minds of Dr Scull and myself as to the existence of what for want of a better term, we have called a low grade "edema" in many cases of chronic arthritis of both types.

Another phase of Dr Davis' work which is of interest is the fact that he found the same albumin globulin ratio in arrested cases of atrophic arthritis as he found in cases of hypertrophic arthritis, which is highly suggestive. As pointed out earlier today by Dr Haden derivations of this nature may well have an important significance.

Caution should be exercised in interpreting observations of this sort as in interpreting the significance of agglutination reactions in arthritis but it appears that Dr Davis has opened wider a window looking out upon important phases of the problem which need to be explored further.

DR RALPH H. BOOTS NEW YORK N Y—Dr Davis seems to offer an explanation for the increased sedimentation rate in severe cases of atrophic (rheumatoid) arthritis. He finds a rise in the globulin fraction and a fall in the albumin fraction. If one accepts Dr Scrapper's explanation of the factors influencing sedimentation rates, the rise in globulin fraction could explain the rapid sedimentation rate in these cases. Furthermore, the increase in the euglobulin fraction indicates that it may be the result of infection. He finds the euglobulin fraction normal in cases of hypertrophic arthritis which coincides with the belief that these are of degenerative nature. I believe this is the first work of its kind in these two arthritic diseases. It would be interesting to see the results of similar determinations in gout, here we have increased sedimentation rate in cases we believe not to be due to infection.

DR HOMER F. SWIFT NEW YORK N Y—Certain therapeutic implications are suggested by this paper. In certain types of edema there are definite alterations in the albumin and globulin ratios and a distinct diminution in total plasma protein, and following the taking of a high protein diet there is sometimes a disappearance of edema with the establishment of normal blood proteins. Has Dr Davis pursued his studies far enough to determine whether similar dietetic procedures are beneficial in patients with chronic arthritis?

DR RUSSELL L. HADEN CLEVELAND O—I would like to emphasize the importance of Dr Davis' work in relation to the occurrence of edema in chronic atrophic arthritis. The important point so far as edema is concerned is the relation between albumin and globulin in the blood plasma. With such changes as Dr Davis describes there is necessarily a lessened osmotic pressure of the blood proteins and so an increased tendency to the development of edema. As Dr Boots has said this might be urged as proof that chronic atrophic arthritis is infectious in type. I have been interested in the relation of albumin to globulin in thyroid

disease. In hyperthyroidism, presumably not due to infection, our findings are almost identical with those reported by Dr. Davis for atrophic arthritis.

DR. S. C. WOLDENBERG, WASHINGTON, D. C.—In the past three years we have run in the Veterans' Hospital at the Bronx, N. Y., over 300 cases of hypertrophic arthritis, and we have found that many of the patients have a normal sedimentation rate. Three weeks later some of these patients had bronchitis or other respiratory conditions; the sedimentation rate was then increased. It has been stated by some that an altered sedimentation rate is an indication for sulphur therapy in the treatment of arthritis.

It may be of interest to state that we have studied the amino acid content of blood in 27 cases of arthritis and have found none of them deficient in any of the amino acids, except the cystine.

DR. DAVIS, JR. (closing).—In answer to Dr. Swift's question, we have done some urinary studies to see if there was an albuminuria that would account for the fall in albumin in the plasma. I have not done enough to really say. I have given a number of these patients a very high protein intake. It does not harm them and several have been benefited. We are working on that line now to see if it has some relation to the change in the albumin fraction.

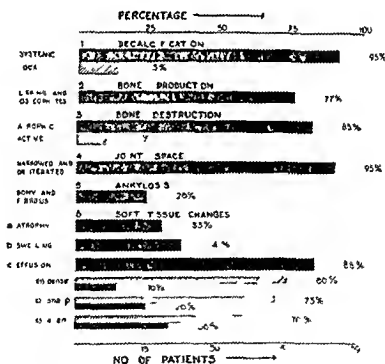
# A STUDY OF THE ROENTGENOLOGIC FINDINGS IN VARIOUS TYPES OF CHRONIC ARTHRITIS

## ABSTRACT

G DOUGLAS TAYLOR, M D, A B FERGUSON, M D, HAIG KASABACH, M D,  
AND M H DAWSON, M D, NEW YORK, N Y

TO CORRELATE the clinical and roentgenologic findings in various forms of chronic arthritis, 163 cases have been studied 52 of rheumatoid arthritis, 32 of osteoarthritis, 12 of gout, 11 of gonorrheal arthritis, 32 of tuberculous arthritis, 12 of Marie Strumpell spondylitis, and 12 of Still's disease

TABLE I RHEUMATOID ARTHRITIS



Among the special roentgenologic characteristics studied were systemic, regional, and local decalcifications production of bone,ipping and osteophytes, active and atrophic destruction of bone, narrowing of joint spaces, bony or fibrous ankylosis, and atrophy swelling or effusion as indicated by soft tissue shadows No single roentgenologic feature is diagnostic for any one type, as the majority of these alterations are common to all types of chronic arthritis, but each type tends to present a characteristic combination of findings that constitutes its own pattern It may be necessary to examine more than one articular region to find these characteristics, and it is recommended that the hands feet knees and lumbar spine be routinely examined by roentgenograms, regardless of the joints of which the patient complains

A study of soft tissue shadows, frequently overlooked, is of utmost importance in the differential diagnosis. Roentgenograms may show little or no change in the early stages of rheumatoid, osteo-, gouty, or tuberculous arthritis, or Haddad-Stumpell spondylitis. In the early stages of gonorrheal

TABLE II OSTEO-ARTHRITIS

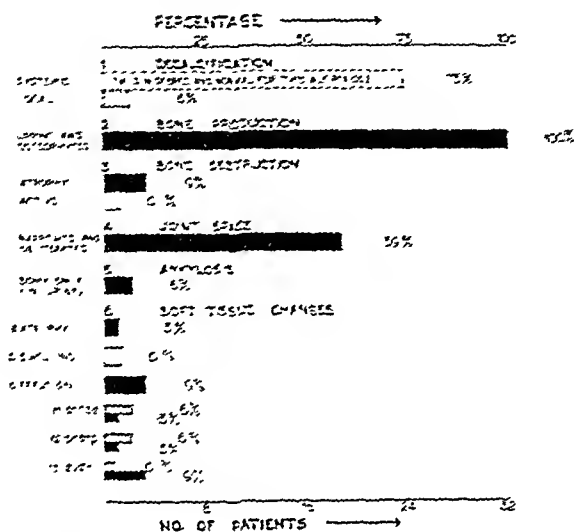
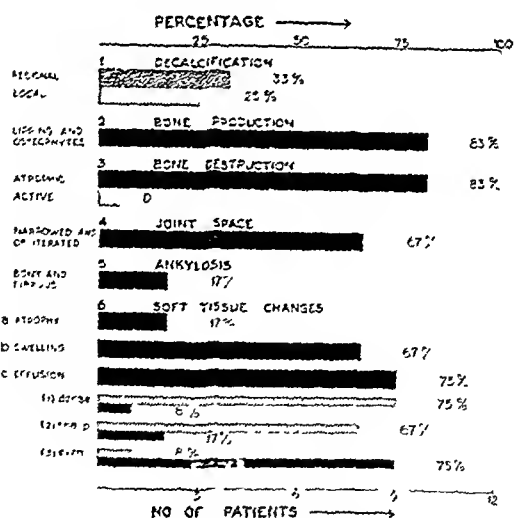


TABLE III GOUT



arthritis, however, roentgenograms are frequently of the greatest assistance in diagnosis. severity of the the roentgen six weeks' d months' dur  
 entgeni should be told at least the duration and  
 ympto order to make a rational interpretation of  
 shadow a appearance of a gonococcal arthritis of  
 ay elo. mble that of a tuberculous joint of six

Our roentgenologic studies indicate that rheumatoid arthritis and osteoarthritis are distinct entities. Rheumatoid arthritis cannot be divided into subgroups by roentgenologic examination. Rheumatoid arthritis, Still's disease, and Marie-Strumpell spondylitis present the same characteristic group-

TABLE IX GONOCOCCAL ARTHRITIS

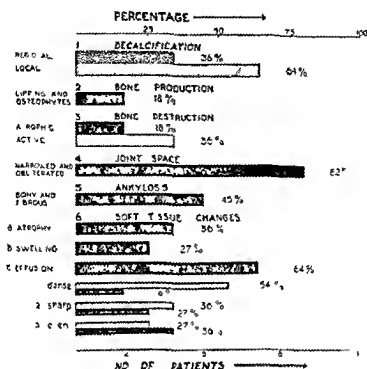
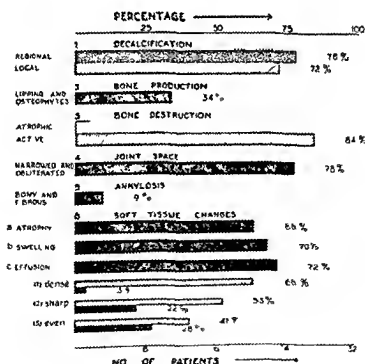


TABLE X TUBERCULOUS ARTHRITIS



ing of the roentgenologic findings, a correlation which supports the clinical conception of the three processes being different manifestations of the same disease. Marie-Strumpell spondylitis and hypertrophic spondylitis (osteoarthritis of the spine) present distinct and different roentgenologic appearances. Localized areas of atrophic destruction of bone ("punched-out areas")

are seen as frequently in cases of atrophic arthritis as in gout. Roentgenograms properly interpreted are a most valuable aid in the differential diagnosis and prognosis of the various types of chronic arthritis.

TABLE VI

## MARIE-STUMPPELL ARTHRITIS

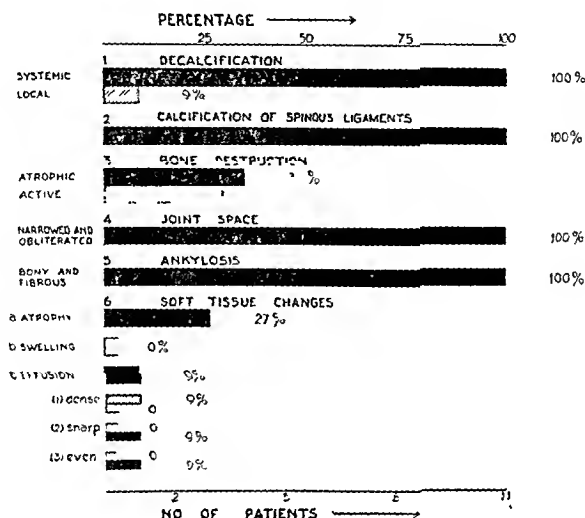
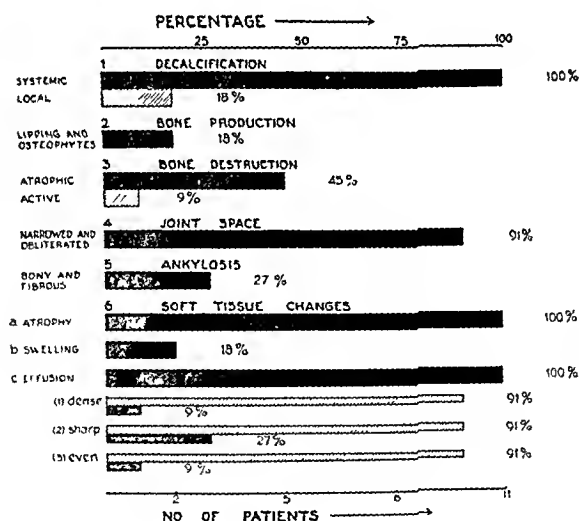


TABLE VII

## STILLS' DISEASE



## DISCUSSION

DR. JOHN G. KUHN, BOSTON, MASS.—This careful study of the roentgenologic findings in various forms of chronic arthritis is most commendable, but all such groupings of characteristic alterations in roentgenograms have definite limitations. While the typical patterns described are seen in the majority of cases at certain stages of the disease, the roentgenologic changes of the joints and their surrounding structures can be modified greatly by repair of the pathologic changes, by a return to something approaching normal function of the joint, and, to a lesser extent, by a diet rich in minerals required in bone

formation. The apparent effusions described by the authors are often simply capsular and synovial thickenings, as operations have repeatedly demonstrated. In roentgenograms they are frequently indistinguishable from effusions. I have been unable to differentiate between the calcaneal spurs seen in gonorrheal infections and those found in cases in which faulty statics of the lower extremity is apparently the only cause. With the other findings we are in entire agreement.

DR A. B. FERGUSON, New York, N. Y.—May I enlarge on some of the roentgenologic features brought out by Dr Taylor. We studied forty-nine roentgenologic characters. Each one had a particular meaning to us. Our greatest difficulty is in carrying that meaning over to you. Each term we used has a technical roentgenologic meaning. For instance, the word effusion as used by us covers whatever is within the capsule, whether free material, granulations, or anything else. They all cast the same shadow roentgenologically. May I clarify our meaning about a few things. First decalcification. Bones become less calcified with age. That is what is seen in hypertrophic arthritis (degenerative arthritis or osteoarthritis). When we speak of an abnormal systemic decalcification, we mean that the decalcification is not commensurate with the patient's age. In fact, we have made a roentgenologic diagnosis of degenerative arthritis in a case in which the patient was twenty-eight years of age, solely because of the decalcification in the hands and feet. Local decalcification is a circumscribed decalcification limited to some particular portion of the bones or to a group of small bones such as those of the wrist. Soft tissue shadows are not analyzed sufficiently in roentgenologic literature. It is noted that some mass of soft tissue, which is called swelling or effusion is present, but the detailed characteristics of its appearance and distribution are not studied. Their consideration is of importance because it is the character of these early changes in the soft tissue which enables one to recognize early lesions in cases in which there are no other changes. The characteristic outline of the swellings of the soft tissues and their distribution should be noted. When one considers a certain feature as typical of a disease, one implies that in any case of that disease, the roentgenographic appearance of some joint should conform to that particular finding, even though the appearance of other joints differs. In cases of rheumatoid arthritis there may, for example, be a high percentage of bone destruction, or lipping, but this may not be a typical feature of the disease. Lipping at the great toe may have nothing to do with rheumatoid arthritis. I believe that the feet, hands and knee joints should be examined roentgenographically in every case of obscure arthritis regardless of the joint affected, in order to obtain a general view of the constitutional changes produced by the disease.

DR JOSEPH A. FREIBERG, Cincinnati, Ohio.—The impression that roentgenograms show punched out areas of bone in early as well as in advanced cases of gout is not true. It is important to remember that patients with mild or even moderately severe gout may show no roentgenologic changes whatsoever.

DR J. A. KEY, St. Louis, Mo.—I am glad to see roentgenologists paying attention to swelling of soft tissue and hope they will be able to differentiate between various types of swelling. Swellings of soft tissue are the first evidences of disease in joints and may be present when the bones appear normal. The more I study roentgenograms, the more I need to know about the clinical history of the case. Phenaster showed that narrowing of the joint space was a late finding in tuberculous arthritis because the bearing surface of the cartilage is not absorbed while in neuter arthritis it is absorbed early. In gout, the punched out areas are the result of subchondral deposits in the bone. In neuter arthritis and gonorrheal arthritis the punched out areas are caused by erosion of the cortex of the bone. They occur around the margins of the cartilage and are the result of invasion by the diseased tissue. In considering atrophic bone one should know the duration of the abnormal state and how much circulatory change has been present during that time. Any process which interferes with the circulation and local heat will cause atrophy of bone. Last, one should know how much strain the bone has been subjected to, because lack of function from immobilization or from pain may explain the atrophy.



DR. RALPH PEMBERTON, PHILADELPHIA, PA.—At the risk of appearing somewhat divergent, I would like to call attention to the importance of extending, to a greater extent than now exists, a better knowledge of the gross morbid anatomy of arthritis to the various groups of surgical and other specialists who are working on the fringe of the arthritic problem. Without any reflection on the excellent paper which we have just heard, this desideratum is perhaps nowhere more important than in the field of radiology. A considerable experience has taught me that most radiologists who see an overgrowth of bone make a diagnosis of "hypertrophic arthritis," and if overgrowth is absent the diagnosis becomes "atrophic arthritis." The time has come for a more informed type of scrutiny. There are about 200 of us here today who will shortly return to our respective places of work. I hope that representations will be made by all of us that a demand exists for fuller knowledge of the pathology of the disease on the part of those whose activities touch the arthritic syndrome.

DR. HOMER F. SWIFT, NEW YORK, N. Y.—What do the authors consider the minimal number of roentgenograms which would be required to make a correct differential diagnosis?

DR. M. J. SHAPIRO, MINNEAPOLIS, MINN.—In what percentage of cases is the roentgenologic diagnosis in accord with that based on clinical, bacteriologic, and other laboratory findings? Have any roentgenologic changes been found in joints involved in rheumatic fever, especially in those joints in which there has been long-continued effusion? Can a gonorrheal arthritis be distinguished roentgenologically from ordinary septic arthritis? In what percentage of joints examined roentgenologically can a definite diagnosis be made?

DR. M. H. DAWSON, NEW YORK, N. Y.—I feel that roentgenologic diagnosis is just as reliable, if not more so, than the clinical diagnosis of the arthritis.

DR. G. DOUGLAS TAYLOR, NEW YORK, N. Y.—The diagnoses and analyses of the two roentgenologists, Drs. Ferguson and Kasabach, were made without either of them knowing anything about the clinical histories. We made separate clinical studies, presented the roentgenograms to them, and checked their reports. We then checked the cases by various clinical and laboratory methods. In the Presbyterian Hospital, Drs. Dawson and Boots studied the cases. In the New York Orthopedic Hospital, postoperative examination of sections was made in most of the cases. Consequently, we thought we could defy anyone who disputed the diagnosis in what we called "typical cases." We found that the roentgenologic diagnosis in these "typical" cases agreed with the clinical diagnosis.

The roentgenologists were told the duration of the symptoms, the severity of the disease, and whether the joints had been used, or whether function had been interfered with. We found "punched-out" areas of atrophic bone destruction just as frequently in cases of atrophic arthritis as we did in cases of gout. We agree with Dr. Freiberg that in early gout there may be little or no change in the roentgenograms. In this study we included only the more advanced cases of gout.

## HOME TREATMENT OF CHRONIC ARTHRITIS BY PHYSICAL THERAPY

JOHN S. COULTER, M.D., CHICAGO, ILL.

NO PROGRAM for the treatment of chronic arthritis can be considered complete unless physical therapy is used. The best physical agents are obtained easily, i.e., heat, water, massage, physiologic rest, and exercise. These are used as adjuncts to other forms of treatment. Under direct observation the capillaries of the patients suffering from chronic arthritis show a markedly diminished peripheral blood flow. The therapeutic value of heat, massage, and exercise is to increase blood flow. Therefore it would seem a waste of the patient's time and money to limit physical therapy to one hour three times a week. If physical agents are used, they should be prescribed definitely by the physician. The patient and some member of the family should be instructed in their use at home. Thus the physician can secure for these patients somewhat the same treatment with physical therapy at home that they would get in a hospital or sanitarium, resulting in a considerable reduction in the cost of medical care for the patient and also helping the physician to keep control of the patient over the long period necessary for treatment.

The detailed procedure to be followed, as well as the objectives to be attained, should be carefully explained by the physician during the first visit to the patient. This is quite essential to secure the necessary intelligent cooperation. Usually the patient is shown that in the *Primer on Chronic Arthritis* issued by your association there are eight headings under treatment, and that physical therapy is an adjunct under six of these divisions. Then the use of each physical agent is carefully explained.

### EXERCISE

This program is always started with a careful explanation of and a prescription for body mechanic exercises because it is believed that a most important part of the treatment is to remove mechanical handicaps and to maintain the best possible body mechanics.

The following statement by Osgood<sup>1</sup> is read and explained to the patient in detail: "Atrophic arthritis is much more common in the young, thin, ptotic type of person. The 'set up' is inefficient. Their muscles are poor, their thoracic cages are narrowed, their diaphragmatic excursion is small, their abdominal viscera are sagged, the weight bearing lines of their joints are not true, muscle tonus is hard to maintain because the center of gravity is disturbed. They are fatigued. Their body mechanics is wretched. If bad body mechanics can be converted into good body mechanics, if inefficient 'set ups' can be changed into efficient ones by positional rest periods, exercises, and temporary supports, if more room can be provided in the thoracic cage for heart and lung action, if the diaphragmatic pump can be set working again on the abdominal veins and viscera, if then symptomatic joints can have their normal weight bearing thrusts

restored; then there would seem to be inevitably less wear and tear, a higher circulatory coefficient, more nearly normal alimentation, less fatigue and a better chance for the defense mechanisms of the body to overcome whatever bacterial or chemical toxins are being elaborated, which presumably are or will be responsible for the symptoms in the joints.

"In hypertrophic arthritis, the same principles of improving faulty body mechanics obtain. With its older age incidence, the remodeling of the body mechanics is harder but, fortunately, there is usually less remodeling to be done." The patient is often asked to read certain indicated parts of the recent book *Body Mechanics* by Goldthwait and others.<sup>2</sup>

The patient and some interested member of the family are instructed in the use of body mechanic exercises. The instructions are on mimeographed sheets. These exercises are based on the development of a firm, flat lower abdomen. The position desired is standing so that the weight falls well forward on the outer borders of the feet, the lower abdomen pulled in and up, the back flat, the head up, and chin in. The body should be stretched tall, without being rigid: the shoulders and chest will then fall into their own balance line. The physician should direct carefully and check over the performance of these exercises every week, adding new ones and leaving off the easier ones as the patient improves.

For local joint conditions during the acute stage, deformity must be prevented by rest in proper supports, but painless active motion is encouraged. These joints should never be manipulated by passive exercise, for swelling interferes with the circulation which is already poor and the diseased tissues are traumatized. During the subacute stage there are several forms of exercise that eliminate the weight of the extremity during movement and are helpful in allowing the patient to translate even minimal muscle power into active motion. In Gaenslen's sling suspension method the patient can exercise frequently for brief periods in the pain-free range. With a homemade tank in the average home the buoyancy of the water eliminates gravity and the warmth of the water relaxes the muscles and accelerates the blood flow.

After the acute and subacute stages have passed, free and resistive exercises are given on prescription by the physician. There should be a mimeographed sheet of exercises to be used for each joint. The best result in the restoration of function in stiff joints following chronic arthritis is not from manipulation but from the gradual use of these joints by directed exercise and occupational therapy.

In certain cases exercise apparatus is useful. Directions may be obtained for the construction of stall bars for arm and leg exercises, a shoulder wheel and abduction ladder, a Kanavel hand table, and stairs with rails for stair climbing exercise.

Rest and exercise are essential to the function of a joint. Motion should be encouraged in all stages of the disease, but this motion should be well within the fatigue limit.

Exercise for a single joint is monotonous. The human body is more than a machine and the formal repetition of a movement either with or without ap-

paratus is not of maximum therapeutic value in increasing the amount of movement in a stiff joint or as an integral part of a larger coordinated movement. There is no psychologic stimulant for personal incentive or sustained effort.

Occupational therapy may be applied at home under the direction of the physician and with the help of the family. It is inexpensive and very effective if the patient is willing to cooperate. For example, refinishing wooden furniture involves constant squatting and raising as the rings of a chair and other parts are sandpapered and finished. This bending and straightening of the leg while at work offers exercise while the patient is thinking about his job. He is conscious not only of the exercise but is also inspired by his work. In working at home the patient and his family will take an interest in this form of exercise and during the events of the day will discover other normal tasks which will provide opportunity for the repetition of an exact motion. This is an important factor, for it does away with the more or less unconscious effort to save the affected part. Again definite directions will impress the patient with the surgeon's interest and repay the time taken in the functional restoration gained by the patient.

#### HEAT

Heat is applied locally over an arthritic joint to increase the local circulation. It has been shown that the quantity and quality of this heat must be prescribed by the physician. Landis<sup>7</sup> has shown that the capillary pressure in the arterial side is 32 mm mercury and 12 mm in the venous end. This pressure rises to 60 and 45 mm at a temperature of 42° C. Drury and Jones<sup>8</sup> observed that edema formation is two to five times greater at 42° C than at 16° C. Starr<sup>9</sup> found that 30° to 35° C were the best temperatures to secure an increase in circulation and relief of pain in circulatory disturbances of the extremities. Therefore it is believed that heat should be prescribed by the physician to secure a definite temperature for a stated period.

Breiman<sup>6</sup> confirmed Sonne's work that during the applications of incandescent radiation of maximum tolerance the subdermal temperature at a depth of 0.5 cm was 47.7° C, while with infra red radiation at the same depth the temperature was 41.7° C. Therefore lamps which emit luminous and short infra red rays are more effective to increase the temperature of the subcutaneous tissues than the ordinary infra red generator which predominates in long infra red rays. It is important for the physician to prescribe the kind of radiant heat to be applied.

The patient with chronic arthritis should be given directions for the local application of heat. A useful electric lamp baker can be made for about \$5.00.

Associated with massage electrical muscle stimulation of the graduated contraction type is of great value. The excellent results are due to the direct stimulation of the muscles causing contractions as well as the mechanical effects due to the direct movement of the joint. It has been shown that these contractions are not followed by the production of lactic acid as occurs when muscles are voluntarily contracted. Therefore muscular contraction of this kind partakes more of the nature of massage than it does of active exercise.<sup>7</sup>

An apparatus giving a sinusoidal faradic current has been devised that can be made for \$5.00. In certain cases this apparatus is sent home with the patient and instructions given to a member of the family in its use.

In addition to this local heat the application of heat systemically is of value. There has always been a popular belief in this application of heat for elimination, but Pemberton<sup>8</sup> believes this value to be limited and not the only one. Some of the other effects are the increased circulation and metabolism induced. These effects are increased if following the general bake or bath a Scotch douche is given.

#### HYDROTHERAPY

In the use of water in the treatment of arthritis the Scotch douche and cold shower after a bake furnish a "metabolic and circulatory whip" if properly used. Where the proper apparatus is not available, a sheet bath is a valuable substitute. For home use again the patient should be given definite instructions for its use. For the generalized application of heat the hot bath may be used at home according to the directions given by Cecil.<sup>10</sup>

For local applications of heat by water a homemade whirlpool bath is often valuable. The whirling of the water under pressure and the air intake enable the water temperature to be increased to 110° and 120° F. The rapidly moving water and air bubbles also give an efficient form of gentle massage. Joint motion that could not otherwise be tolerated may be given while in the bath.

Contrast baths using hot water followed by cold are not used, as it has been noted by direct observation of the capillaries through the microscope that the capillary circulation is not increased by contrast baths as efficiently as with hot baths.

#### MASSAGE

There seems to be a deficient blood supply to arthritic joints. In the early stages of the disease when there are no joint signs and only intermittent joint symptoms, increasing the local blood supply to the joints by heat, massage and active exercise may restrain or prevent permanent tissue changes in the joint.

Pemberton<sup>8</sup> has shown that the effect of massage in arthritis is to open vascular channels and increase the circulation. If this is so and there is a deficient circulation to these joints, it must follow that massage must be given at frequent intervals. For this reason we have our patients bring in some interested member of the family for instruction in the use of massage at home, as it is useless to give massage three times a week and no more. Every physician should be able to prescribe massage as he would drugs. It is dangerous to turn a patient with arthritis over to a technician with only directions to give massage. Heavy massage over an arthritic joint may cause a marked local reaction. An arthritic case should have gentle kneading massage above and below the affected joint and no massage over an acute joint. When the joint becomes subacute, superficial stroking massage may be started over the joint in addition to the kneading massage above and below the joint. This should be preceded by at least fifteen minutes of heat. Massage should be given for about ten minutes twice daily. The details of the technic of massage are given in the *Handbook of Physical Therapy*.

## REST

If chronic fatigue is one of the chief factors in all arthritis, certainly rest is one of the most important agents in the treatment of the disease. Swain and Kuhns<sup>9</sup> state that fatigue is not only a subjective symptom but the physical signs of it are also present, for example subnormal morning temperature, poor pulse, vasomotor instability, low blood pressure, lowered metabolism and poor muscle tone. They believe that fatigue of this kind is due to disturbances of the normal physiology of the organs in the thorax and abdomen, and has its effects chiefly through the circulation and sympathetic nervous systems as is shown by cyanosis of the lips, cold hands and feet, localized blanching, skin changes, and deranged heart action.

Rest should be prescribed with as much discrimination as any drug. Usually the rest should be in bed for certain periods daily or continuously. At first, after each meal a pillow is placed under the shoulders, the hands are placed under the head, and a pillow is placed under the knees. This raises the chest and abdomen, hyperextends the dorsal spine and results in abdominal breathing with better use of the diaphragm.

After a half hour the face prone position is taken and radiant heat is applied to the spine to stimulate spinal circulation. These periods are given after meals whether the patient is in bed continuously or is ambulatory. If continuously in bed these periods are continued until exercises and proper supports enable the patient to continue this correct position when up. They are then allowed up for increasing periods.

Rest is also an important agent in the local treatment of the affected joints. Rest and exercise are most important in the function of a joint. This rest should be in a position best adopted to prevent strain or contracture, thus preventing deformities. These positions have been described by Swain and Kuhns.<sup>9</sup> The prescription of local rest illustrates the necessity of having the cooperation of an orthopedic surgeon in the treatment of chronic arthritis with physical therapy.

## CONCLUSIONS

In chronic arthritis sufficient time for adequate treatment is fundamental, and physical therapy is an important adjunct in treatment. These physical agents can be used by any physician in any place, as they are cheap and easily obtainable, but to secure the best results they must be carefully prescribed and used under the physician's directions, not only in the hospital and office but also for longer periods at home by the patient assisted by some member of the family.

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## DISCUSSION

DR. FRANK R. OBER, BOSTON, MASS.—Hospital physiotherapy under our present set-up for managing arthritic patients is impossible, since there are too many patients who have arthritis and not enough hospital beds to care for them. We have no mechanism whereby home treatment may be given, and there are not enough physiotherapists at present to instruct members of the family. Furthermore, very few physiotherapists are interested in treating arthritic patients and the average practitioner does not know enough about physiotherapy to carry out a well-balanced plan. In Massachusetts about 70,000 people suffering from arthritis receive no treatment whatever.

Under present conditions, the management of acute joints by home treatment is a dangerous occupation for either the physiotherapist or the family doctor. These arthritic patients must be handled very carefully. The doctor should understand how much exercise is necessary to promote the function of their joints. If they are to be badly treated, it would be wiser to let them alone. I do not believe anyone in the home can be instructed in the proper use of electricity, and it is doubtful in my mind if electricity has any great value. Many people think heat, electricity, and massage are going to work a miracle. These are only a very small part of treatment. If it comes to a choice between heat, electricity, massage, and exercise, throw the first three out of the window and use exercise.

The program which Dr. Coulter suggests is ideal but impossible to follow out in the average hospital in the United States, unless such a hospital has a physiotherapy department. If we keep hammering away at various schemes for managing arthritic patients, there is no doubt that ultimately home physiotherapy can be used more effectively. One way home treatment might be brought to the patient is to organize a committee of doctors, nurses, and physiotherapists interested in arthritis, who could go out in teams, meet local physicians, and go over the situation. A visiting nurse or physiotherapist could be appointed to cover certain communities and to teach home treatment. It is only through a well-organized plan that we are going to be able to treat this large group of patients.

There should be an organized plan so that physiotherapists, family doctors, and people in the homes can receive an adequate amount of instruction in the care of joints. It is only through such an arrangement that we will be able to treat the majority of arthritic patients. What should be done is easy enough to suggest, but how to accomplish it is another matter.

The great tendency in treating arthritic patients is to give them heat, massage, or electricity. Too much of any of these is bad medicine. Members of the family can help the patient with his exercises after the acute inflammation has subsided. Exercises should always be active or active plus passive. Passive motion in an arthritic joint will increase inflammation in synovial membrane. Scar tissue and ankylosis may result. Heat is used at home to a large extent but I believe it is bad therapy, since heat will not cure arthritis. There is too much buying of bakers and diathermy machines by people who do not know how to use them or what the dose should be. I have seen patients who have had home baking to such an extent that their limbs looked as if they had been covered with blobs of chocolate.

DR. CHARLES L. LOWMAN, LOS ANGELES, CALIF.—Very often too great a jump is made from rest to exercise. When muscle atrophy exists, there should be a carefully graded progress from bed rest to active exercise of involved joints.

This can be best accomplished in water. The absence of weight load on the joints by the elimination of gravity makes possible a greater amount of muscular activity with its beneficial effect on nutritive, circulatory, and eliminatory processes, without the intraarticular pressure incident to work done out of water.

Greater range in the painless are even in a subacute joint can be gained in active movement of an assistive type when the advantage of the lift of buoyancy is used. For the advantage of general peripheral dilatation of vessels obtained by submersion in a warm bath can be supplemented by exercise, preferably, of course, in a therapeutic pool under technical guidance, but at home a limited amount of leg and arm work can be obtained by a fifteen to twenty minute period in a warm saline bath with less risk. Sea salt and commercial epsom salt can be used at very nominal expense.

Our practice in pool therapy is a sequential one. First, movements of the uninvolved areas of the body, especially abdominal wall muscles, are prescribed. These are important in order that a proper degree of resistance to the action of the diaphragm may be obtained, as the effect of any pump depends on the amount of compression as well as the force and range of the plunger stroke. Second, two sets of muscle groups are always activated for any movement of a part. Intrinsic muscles activate a given joint but their base of action must be concurrently fixed by extrinsic stabilizing groups. In arthritis both sets are atrophied from disuse or continued spasm. Consequently in subacute cases you can at least tone up the extrinsic muscles before the time when it is advisable to begin on the intrinsic ones. As these are chiefly trunk groups there is the added benefit of the improvement of spinal, abdominal, and respiratory function essential in combating the systemic part of the disease. Third, the joints involved may be exercised, never passively but actively or by active passive movements. As the range or arc of motion even in an acutely painful joint is much greater in warm water, the reparative influence is more rapidly obtained and the mental fear complex which has been initiated previously is absent or rapidly subsides. Fourth, weight bearing can be graduated in water when the right kind of depth pools are available. The patient who has not been able to stand for a long time actually sees himself upright and his legs go through the greatest possible amount of steppage, with a weight load decreased to only a few pounds. The weight dosage is graded by gradually proceeding to shallower depths.

I agree with Dr. Coulter that with care on the part of the doctor and intelligent co-operation on the part of the patient and some member of the family, some home regime is not only possible but very desirable. We require patients to take enemas at home, to adjust the diets according to orders, to take sun treatments, to follow exercises after fractures, to douche their noses and gargle their throats for nose and throat affections, so why should we not expect them to carry out simple orders for physical therapy?

DR. PHILIP S. HENCH, ROCHESTER, MINN.—May I express complete approval of Dr. Coulter's views. Questioning a large number of arthritic patients, I found that 75 per cent had consulted osteopaths or chiropractors. The reasons they gave for such defections from orthodox practice were that their physicians either gave them no physiotherapy or gave it to them haphazardly, "not often enough to keep me relieved," or because physiotherapy in the physician's or professional physiotherapist's office was too expensive, and more costly than they could get it elsewhere. We cannot expect much from physiotherapy applied only twice a week, or even daily for only twenty-one days the usual "course." It is ridiculous to expect best results from physiotherapy used yesterday but not today or tomorrow. It is as if we wore a raincoat yesterday when it was raining hard but not today when it is pouring and wonder why we are still wet.

Few patients, however, can long afford professional physiotherapy more than two or three times a week. They should be permitted, indeed urged, to supplement such service with home treatment. In this country and in Europe, there are very few spa physicians who make a real effort to teach the departing patient home physiotherapy in order to project into the patient's home environment at least some of the benefits of the spa. For some years it has been our custom at the Mayo Clinic to give every arthritic patient and where possible his relatives also, as a supplement to other measures, detailed instructions in simple and harmless methods available for home use. A physiotherapy highbrow may scorn such procedures as inadequate, but they are a lot better than nothing, and fill the need felt by the patient who has sought relief from a hot bath, a strip of flannel and a hot iron, a firm oven or a bag of heated sand, salt or oats. If his physician does not tell him of better methods, he will continue his amateur efforts or get help from the cultist.



Now for home physiotherapy we have the approval of Dr Coulter, chairman of the Council on Physical Therapy of the American Medical Association. Although home treatment entails a little danger at the hands of physicians and patients inadequately instructed in its principles and details, surely safe methods can be taught by intelligent physicians and technicians to intelligent patients.

DR RICHARD KOVACS, NEW YORK, N. Y.—May I call attention to the menace of advertising diathermy machines for home treatment over the radio and by mail. This is evidently a profitable venture for at present no less than four firms are advertising in New York with special emphasis on good results in arthritis. This situation is due to the laxity of control over radio advertising and unfortunately also to the attitude of some physicians in condoning this dangerous practice. We are combating it by spreading intelligent information through the Bureau of Information of the New York Academy of Medicine and the Better Business Bureau. We are confident that this fad will ultimately run its course just as that of home treatment by ultraviolet rays did, but in the meantime it is a real menace and might spread to other localities.

DR COULTER (closing).—I think the family physician will have to be the one to treat chronic arthritis if Dr. Ober figures there are 70,000 arthritic patients in Massachusetts receiving no treatment whatever. The family physician is competent to treat arthritis and to use the simple methods of home treatment. He and the medical student should be instructed in the use of the simple physical agents, such as heat, massage, and exercise. The family physician must direct home physical therapy and can do it very easily with the simple directions we give. The best place to give it is in the home, since it will reduce the cost of medical care which we hear so much about today.

## CHRONIC ATROPHIC ARTHRITIS THE EFFECT OF A HIGH CARBOHYDRATE DIET AND INSULIN ON THE SYMPTOMS AND RESPIRATORY METABOLISM\*

BYRON D. BOWEN, M.D., AND L. MAXWELL LOCKIE, M.D., BUFFALO, N. Y.

THIS study was made primarily to observe the effect of overnutrition on patients with chronic atrophic arthritis who had lost weight excessively. Since there is some controversy concerning the proper carbohydrate content of the diet for patients with chronic atrophic arthritis, and since, in view of this discussion, it seemed desirable to observe the effect of a high carbohydrate diet on such patients for long periods, such a course was selected as the method of obtaining overnutrition. As our observations continued, no contraindications for the employment of such a diet became evident.

Such a study appeared, also, to afford an opportunity to observe the effect of hypernutrition and a high carbohydrate diet during the process of increasing body weight on the respiratory metabolism both with and without insulin.

*Method of Study*—Upon admission the patients were given a high carbohydrate diet (425 to 500 gm. daily) with adequate protein to keep them in nitrogen equilibrium and sufficient fat to bring their total caloric intake from 2,200 to 2,700 calories daily. As far as possible the patients were allowed to choose their own articles of food. The diets were calculated by the same method as is used in estimating weighed diabetic diets, which we thought was sufficiently accurate for the purpose of this study. Food which was not eaten was returned to the diet kitchen and when this was carbohydrate, an equivalent amount of carbohydrate was sent back to the patient in the form of orange juice. When the patients were receiving insulin, orange juice was usually given between meals and also in the evening, excepting when the patient was to go to the metabolism laboratory the following morning. Then if a hypoglycemic reaction occurred, only 50 cc. of orange juice was given. Usually the patient's respiratory metabolism was determined at weekly intervals. This occasionally was not possible because the patient could not be made sufficiently comfortable for a successful test. The expired air was collected in the gasometer for three periods of fifteen minutes each. The air from the first period was discarded and gas analyses made on the others in the Haldane apparatus. It was required that the ventilation volume and the CO<sub>2</sub> percentages of the expired air check rather closely before the test was accepted, so that the effect of hyperventilation could be eliminated as far as possible.

The patients who were able were encouraged to be as active about the wards as they wished, some of them were permitted to leave the hospital for auto rides and to sit in the open air when the weather was favorable.

Most of the patients received some form of physiotherapy—ultraviolet light, infra red light treatments, and gentle massage. If they had pain, acetylsalicylic

\*From the Buffalo General Hospital and School of Medicine, University of Buffalo.

acid was given. A few of them required sedatives at night. Most of them received *Streptococcus hemolyticus* (AB<sub>12</sub>) vaccine in small doses for varying periods of time.

## AVERAGE DIET

Carbohydrate 500 gm. Protein 60 gm. Fat 50 gm. Calories 2,690

Breakfast:		GRAMS
Choice	Cooked cereal (dry weight)	30
of	or	
One	Cornflakes, shredded wheat	30
	Bread (rye, white, or whole wheat)	60
	Sugar	20
	Skimmed milk	120
	10% fruit juice	200
	10% fruit	160
	Jam or jelly	60
	Butter	10
	Coffee or tea	

## Noon Meal:

Choice	Lean meat	30
of	or	
One	Fish (plus 6 gm. butter)	40
	5% vegetable	120
	Baked or boiled potato or rice	90
	Bread (rye, white, or whole wheat)	60
	Buttermilk or skimmed milk	60
	Sugar	26
	Butter	19
	10% fruit juice	200
	Canned fruit	100
	Jam or jelly	30
	Coffee or tea	

## Evening Meal:

Choice	Egg (one)	
of	or	
One	Cottage cheese (plus 8 gm. butter)	30
	5% vegetable	120
	10% vegetable	120
	Potato (baked or boiled)	120
	10% fruit juice	200
	Canned fruit	100
	Bread (rye, white, or whole wheat)	60
	Butter	15
	Sugar	33
	Buttermilk or skimmed milk	120
	Coffee or tea	

## CASE REPORTS

CASE 1.—*History*: M.M., a telephone operator aged fifty-two, admitted to hospital Jan. 11, 1934, and discharged Nov. 14, 1934. She had been well until the onset of arthritis in 1929 which began with pain and stiffness in various joints. Deformity of the wrists and hands had appeared in the past two years. She had not been able to work for about a year. Her loss of weight had been gradual for the past five years from 140 to 106 pounds.

*Examination*: An undernourished woman confined to bed because of weakness and pain. Double dentures. Throat red; tonsils imbedded. Joints: right shoulder, rotation limited and painful. Right elbow, motion painful. Wrists, extensive swelling and limitation of motion. Hands, the phalangeal joints were swollen, deformed and tender to pressure. The patient was not able to make a fist. The hands showed ulnar deviation and atrophy of the lumbricales muscles.

*Laboratory Examinations* Urinalyses, all negative Blood red blood cells 4,600,000 per c mm, hemoglobin 70 per cent (Sahli) Sugar (fasting) 126 mg per 100 cc Sedimentation rate, 30 mm one hour, corrected for cell volume Agglutination of hemolytic streptococcus (AB<sub>12</sub>), positive to a dilution of 1280 of blood serum

*Course* Upon admission a diet of 450 gm of carbohydrate, 60 gm of protein, and 50 gm of fat was prescribed This she ate fairly well, but frequently it was necessary to make up some of the carbohydrate in orange juice, and consequently the protein and fat were a trifle less than that allowed Soon after she entered, a mild respiratory infection developed which persisted for nearly a month, this was accompanied by several short bouts of fever During this period her weight increased slightly

Acetylsalicylic acid or salicypyrine was given as necessary for pain and phenobarbital was often required at night Gradually it became apparent that there was more motion in the involved joints and that she was requiring less medication for pain

Beginning March 12, 1934, 12 units of insulin were given before each meal At this stage she had gained six pounds The dosage of insulin was rapidly increased to 18 units, a point where mild hypoglycemic reactions occasionally occurred Insulin was continued until Sept 7, 1934, when salt solution was substituted At this time her weight was 145.5 pounds She had changed from a bedridden patient to one who was able to be up all day, go for short walks, take a tub bath and care for herself generally Her attitude toward her illness had likewise improved, on admission she was disgruntled and unhappy, now jovial and optimistic concerning the future She had required no medication for the relief of pain since April and none for sleep since July She was discharged in November in excellent condition Her weight was 149 pounds She was requested to follow her hospital diet as closely as possible at home Unfortunately, she was thrown into an unavoidable domestic situation which compelled her to do more work than she could comfortably tolerate Nevertheless, during the past seven months she has carried on with but moderate discomfort Pain has been present particularly in the hands Her appetite has been somewhat reduced and at the present time (May 1, 1935), her weight is 137 pounds

*CASE 2—History* B L, a widow aged 66, admitted April 18, 1933, and discharged July 15, 1934 She had extensive atrophic arthritis which had started two years previously in the middle finger of the right hand In the past year it had involved the toes, knees, hips, other fingers, and the left elbow and shoulder She had had considerable pain and had been essentially bedridden for several months prior to admission Her appetite had been poor, during which time she had lost 40 pounds

During the winter prior to the inception of the arthritis she had had frequent sore throats, but it could not be specifically determined that a throat infection had occurred just before the first joint had been involved Her teeth had been extracted several years previously In July, 1932, she had Bell's palsy which ran a course of several weeks At that time, previous to the development of the arthritis, the laryngologist had reported a nasopharyngitis with questionable sinusitis

*Examination* A considerably emaciated woman who did not appear to be quite the reported age She is badly crippled with typical atrophic arthritis as stated in the present illness Her legs are contracted

*Laboratory Examinations* Urinalyses, negative Blood red blood cells 4,500,000, white blood cells 8,800 per c mm, polymorphonuclears 52 per cent, hemoglobin 70 per cent (Sahli) Blood Wassermann reaction negative Sugar (fasting) 124 and 114 mg, urea nitrogen 15 mg, uric acid 2.8 mg per 100 cc Agglutination, *Streptococcus hemolyticus* (AB<sub>12</sub>), positive to a dilution of 2560 of blood serum Sedimentation rate, 66 mm at the end of one hour

*Course* On admission her weight was 97 pounds During the first fifteen days of her stay, she ingested daily about 360 gm of carbohydrate 45 gm of protein and 61 gm of fat This totaled 2,190 calories, which was considerably less than that prescribed During this period her weight increased to 104 pounds In the next period, sixteen days, she received insulin, 16 units a c and ingested in average of 795 gm of carbohydrate 43 gm of protein, and 57 gm of fat During this period she developed a typical attack of acute follicular tonsillitis which apparently did not produce any change in her joints This did

not cause any weight loss. In the third period, twenty-one days, 20 units of insulin were given before each meal. The average daily diet was carbohydrate 437 gm., protein 41 gm., and fat 60 gm., totalling 2,464 calories. At the end of the third period her weight was 108 pounds. In the fourth period, thirty-nine days, the diet was similar, about 2,500 calories. Insulin, 24 units before meals, was given. Her weight increased to 116 pounds. In the fifth

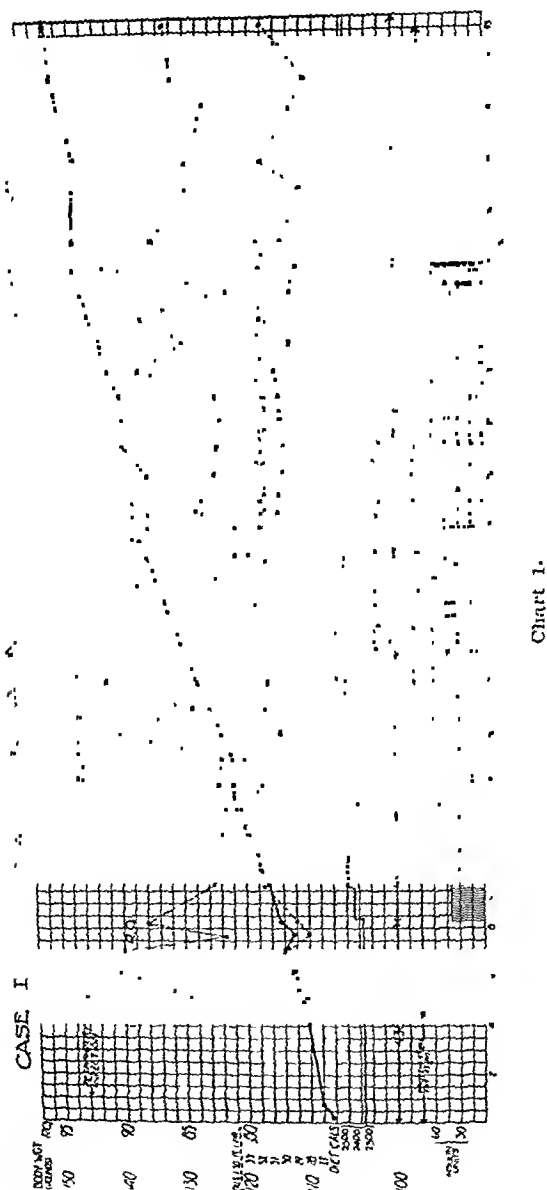
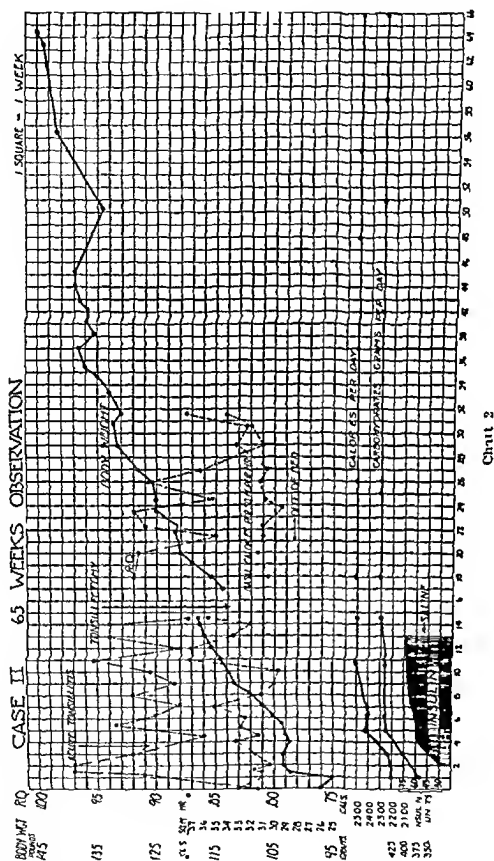


Chart I.

period, nineteen days, only sterile salt solution was injected before meals. Her average daily food consumption was 2,530 calories. Her weight increased to 120 pounds. During her study thus far, there had been slight but definite improvement in her general well-being. She had less pain and could handle herself in bed very much better. On Aug. 14, 1933, her tonsils were removed under a general anesthesia. She was not able to resume her regular diet until August 26, and had lost six pounds. Since her appetite was unimpaired, insulin was

not resumed. By September 18 she weighed more than she did preoperatively. A diet of 460 gm of carbohydrate, 50 gm of protein, and 60 gm of fat was continued up to her discharge on July 18, 1934. Her weight on July 7 was 145 pounds. The pain had gradually reduced and the range of motion had generally increased so that by September, 1933, she was able to get into a chair. She could have walked but for the contractures at the knees.



She required no medication for pain but occasionally received Libralid at night. A survey of her state on Feb 17, 1971 was as follows: "Her back is still painful while lying flat, her left hand and wrist are much improved—no redness or pain on pressure and the range of motion is definitely increased. The left elbow and shoulder are not involved whereas on admission she had about 30 per cent range of motion in these two joints. Right hand and

wrist, less swelling and pain on motion. Right elbow and shoulder, no pain, motion is excellent in all directions. She can reach to her back which was not possible upon admission. Hips, motion is painful while lying on her back, but not so when lying on her side. Knees, there is essentially no change in motion, but they are less painful. Ankles, no swelling—sometimes are painful. She is now able to get around the wards and corridors in a wheel chair." From this time until her discharge in July, 1934, there was less noticeable improvement, and it appeared that the maximum benefit had been attained. She returned to her home until readmission in January, 1935, for the purpose of considering the advisability of operative measures to her knees. Then it was learned that there had been a gradual return of pain and swelling in the right wrist and ankles, particularly the former. While at home her appetite had diminished and she lost ten pounds. She was obviously discouraged. The high carbohydrate diet was again instituted but she took only a portion of it. After two weeks' observation without there being any change in her state, insulin, 20 units a c, was again given. In a few days she developed pain which did not subside with the substitution of beef for pig insulin but did so a few days after the withdrawal of insulin altogether. At the present time (May 1, 1935) when she is allowed to eat what she chooses, her daily food consumption is only 1,600 calories.

**CASE 3—History.** M. N., housewife aged fifty-two, had been in the hospital since Oct. 6, 1934. She had been well up to a year and a half prior to her entrance when "neuritic" pains began in the knees and shoulders. The knees became swollen and tender in November, 1933; involvement of the wrists began in April, 1934. Gradually, the pain, swelling, limitation of motion and deformity increased in the knees and wrists. The ankles and shoulders were affected, but to a lesser degree.

Her maximum weight was 155 pounds eight years ago. At the onset of the arthritis it was 135 pounds. Now it is 104 pounds. She believes that worry over the arthritis rather than loss of appetite accounts for the loss of weight.

Her teeth had all been removed eight years ago. She had had no significant infection recently. A carbuncle which had required excision had occurred on the right arm four years ago.

**Examination.** A somewhat pale, undernourished woman who had atrophic arthritis. The tonsils were the buried type; material could be expressed from them. The joints were as indicated in the history. Otherwise, the physical examination was not remarkable.

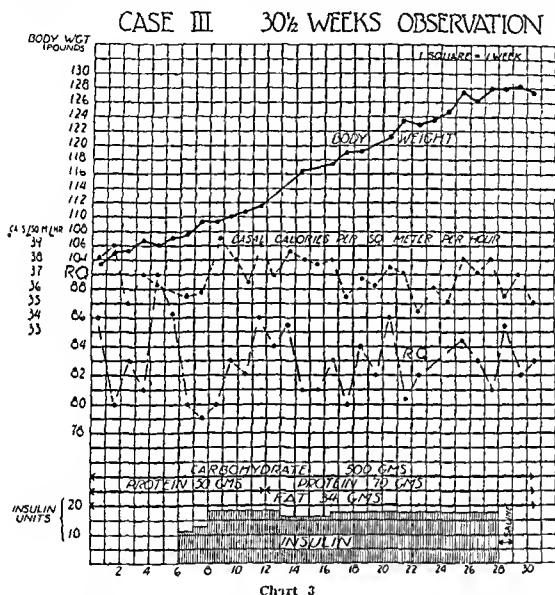
**Laboratory Examinations.** Urinalyses, negative. Blood: red blood cells, 4,100,000, white blood cells 7,600 per c. mm., polymorphonuclears 67 per cent; hemoglobin 70 per cent (Sahl). Blood Wassermann reaction, negative. Sugar (fasting) 121 mg., urea nitrogen, 11 mg. per 100 cc. Sedimentation rate on admission, 39 mm. in one hour, on Jan. 18, 1935, 34 mm. in one hour, both corrected for cell volume. Agglutination with *Streptococcus hemolyticus* (AB<sub>12</sub>), positive up to 1:1280 dilution of serum.

**Course.** Upon admission she was given a diet of 500 gm. carbohydrate, 50 gm. of protein, and 34 gm. of fat. She ingested, with great regularity, all the carbohydrate and nearly all the fat, but fell short of the protein allowance about 5 gm. daily during the period of six weeks before insulin was given. While she was ingesting 2,500 calories daily her weight increased 35 pounds. Insulin was then started, 12 units a c, and gradually increased to 18 units. She had but an occasional hypoglycemic reaction. The diet remained the same for another six weeks during which time her weight increased another 35 pounds. Then for a period of five weeks the protein in her diet was increased to 70 gm. During this period her weight increased 65 pounds. The fat in the diet was now increased to 50 gm. daily. During this period, nearly twelve weeks, the patient gained 10 pounds. The fasting blood sugar just before insulin was stopped was 113 mg. per 100 cc. The blood sugar taken just before the noon insulin dosage was 92 mg. at one time and 106 mg. at another. Three days after the withdrawal of insulin the fasting blood sugar was 121 mg. per 100 cc. and nine days after, the digestion blood sugar was 128 mg. The urine was examined for sugar four times daily for a week following the withdrawal of insulin. No glycosuria was found even though the patient continued to ingest a high carbohydrate diet. During the entire study the patient received her full quota of carbohydrate, since the carbohydrate portion of her diet which was returned to the diet kitchen was brought back

to her in the form of orange juice. The protein and fat contents of her diet were usually somewhat less than the prescribed amount. However, the average daily consumption of food was always in excess of 2,500 calories.

Since Oct 22, 1934, she has received small doses of streptococcus vaccine (AB12), subcutaneously. At the present time she receives three million organisms every ten days. Also, she is being treated with ultraviolet and infra red light along with gentle massage of her joints.

After she had been under observation for a month, it was noted that she had less pain and that her joints had more freedom of motion. After two months she was able to walk to the bathroom with the aid of a cane, and with very little pain. She can now fully



extend the left leg and can almost completely extend the right one without pain. This represents an increase of thirty degrees in the range of extension. Also, she can lift both legs from the bed in an extended position which formerly she could not do. Now, most of the pain and stiffness is in the right knee and right wrist.

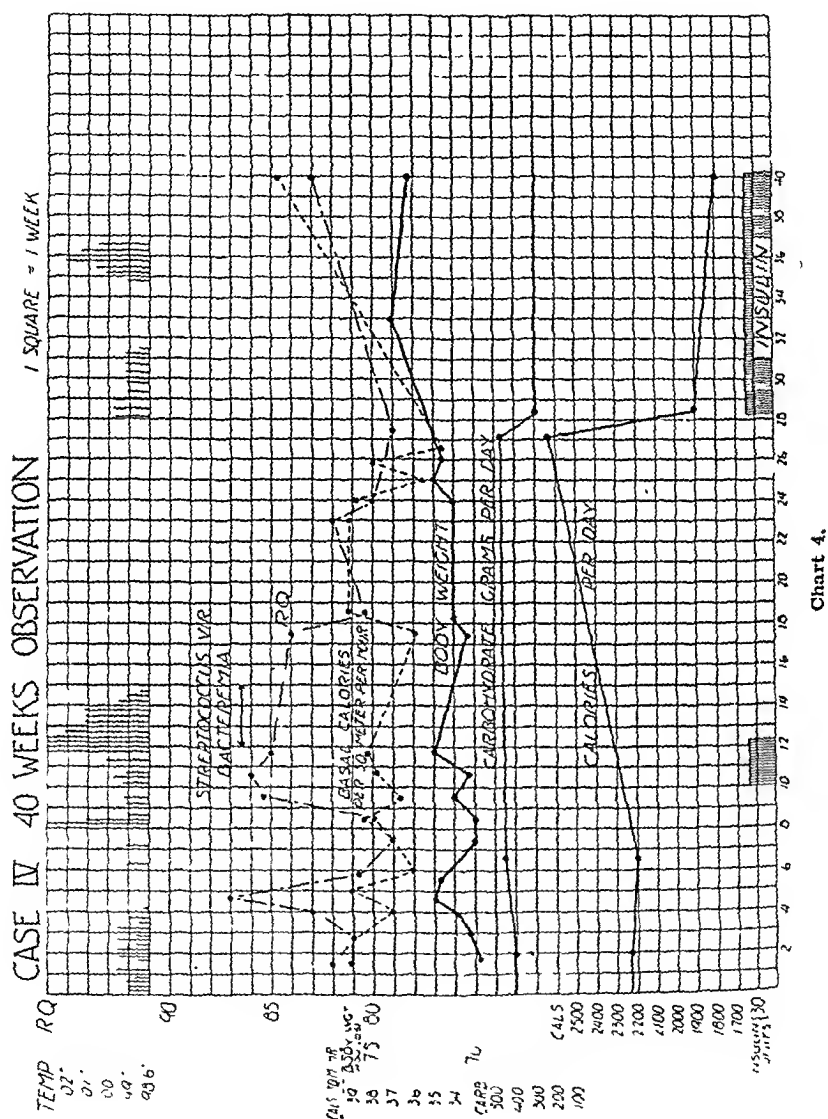
**CASE 4—History** J. G., a schoolgirl aged eighteen, admitted Feb 22, 1934, has been a patient in the hospital since that time. In April, 1932, a month after an attack of scarlet fever, she developed pain, swelling, and redness in the fingers and ankles. This process gradually spread to the other joints of the body including the back, hips, and jaws. Tonsillectomy was done in May 1932 but it had no beneficial effect. Her loss of weight had been 10 pounds.

**Examination** An emaciated young girl whose joints showed the typical changes of advanced atrophic arthritis of the fourth stage. There was a systolic murmur at the mitral



region of the precordium which was not widely transmitted. A few scattered râles were heard at the right supraclavicular region.

**Laboratory Examinations:** Urinalyses, negative. Blood: red blood cells 4,460,000; white blood cells 8,200 per c. mm.; polymorphonuclears 60 per cent; hemoglobin 65 per cent (Tallqvist). Wassermann reaction, negative. Sugar (fasting) 95 mg. on admission, 90 mg. in January, 1935; urea nitrogen 10 mg. per 100 c.c. Sedimentation rate,\* 33 mm., on admission in August, 1934, 60 mm., and in January, 1935, 25 mm. at the end of one hour, all corrected



for cell volume. Agglutination, *Streptococcus hemolyticus* (AB<sub>12</sub>), positive up to a dilution of 1:1280 of serum. Six blood cultures, taken during febrile periods, were all negative except one which was taken during a chill. This showed one definite colony of *Streptococcus viridans*. Agglutinations of blood serum were negative for *B. typhosus*, *B. paratyphosus* A and B, and *B. abortus*.

\*All sedimentation rates were done by the Wintrobe method.

*Course* Her case seemed like a hiccup one from the first, but since no provision could be made for her at home or in another hospital, it seemed necessary for us to keep her under observation. She usually had some afternoon fever and on two occasions, once in April and once in May, she had a sharp rise of temperature following a chill which immediately subsided. Later on, longer bouts of fever occurred.

Upon admission she was given a diet of 450 gm of carbohydrate, 60 gm of protein, and 62 gm of fat. Her appetite was poor and great difficulty was experienced by the attendants in encouraging her to eat. She usually received the full carbohydrate quota of her diet but she appeared to have considerable aversion to protein and fat. Her weight upon admission was 70 pounds. Her best weight was reached on August 13, 1934, when she weighed 74 pounds. Her diet consisted principally of carbohydrate although there were days when she took her full diet. Insulin, 12 units *q.c.*, which was started on Sept. 24, 1934, at the time when her appetite was beginning to want, had no effect. On Nov. 20, 1934, her weight was 73 pounds.

The signs at the right pulmonary apex were watched rather carefully. It became evident that there was increasing activity. An x-ray taken in May, 1934, showed both pulmonary apices to be slightly hazy. By December, 1934, there was distinct cloudiness on the right side. By May, 1935, the right upper pulmonary lobe showed marked density and mottling. Physical signs at that time indicated a rather extensive involvement with probable cavity formation. Her fever which has been present from time to time is now quite regularly present, running up to  $101^{\circ}$  to  $102^{\circ}$  F. in the afternoon.

*Comments* Because of the positive blood culture on one occasion and the mitral systolic murmur over the heart, the diagnosis of malignant endocarditis had been considered. With the signs, however, at the right pulmonary apex it seems probable that she has pulmonary tuberculosis. She failed to gain weight in spite of a rather adequate caloric intake over a long period.

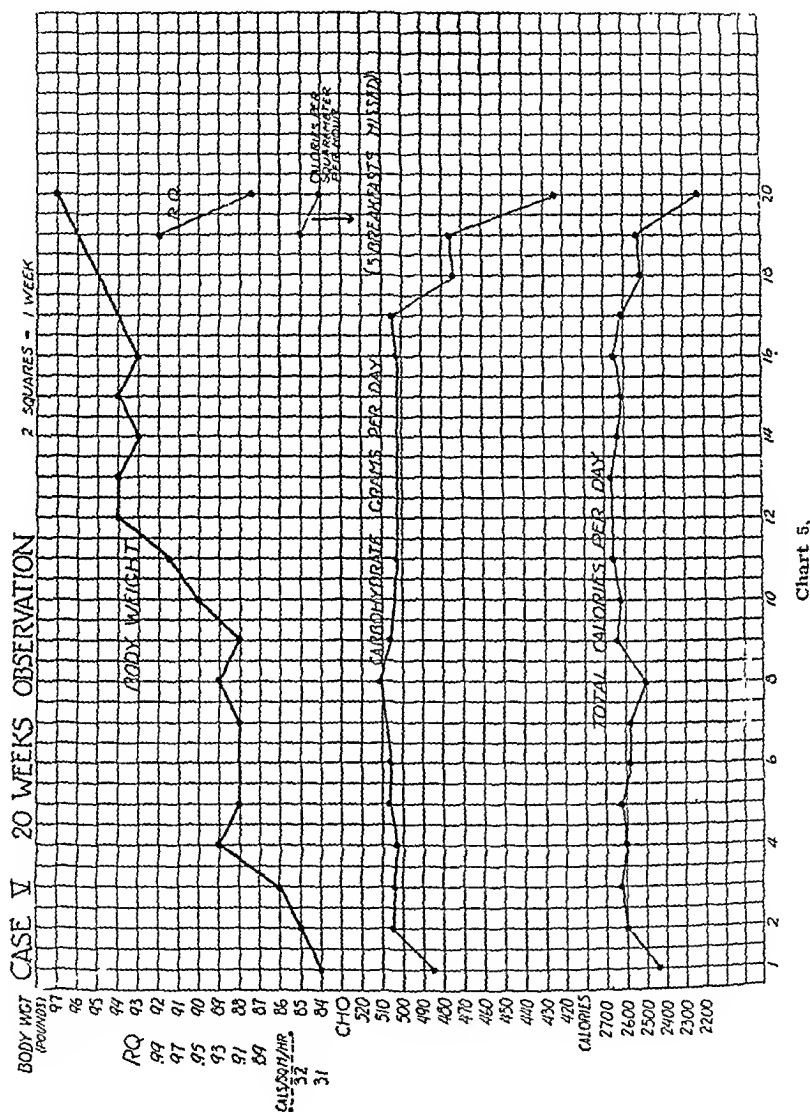
*CASE 5—History* J. A., housewife, age 44, admitted to the hospital May 2, 1934, and discharged Oct. 2, 1934. The arthritis had begun in 1922 involving only the left hip. It had progressed rather slowly. Now both knees, hips, wrists, elbows, and shoulders are affected. The right hip which had been involved for seven years gives her the most distress. During the past ten years she had lost 40 pounds. This she attributed to a poor appetite. The only treatment had been the removal of a few teeth, and occasionally artificial heat had been used.

*Examination* An undernourished woman (weight 84 pounds), who was considerably crippled with atrophic arthritis. Four teeth remained, the gums were infected. The involved joints were swollen, and their motion was reduced. The movement of the shoulder and left hip was particularly limited.

*Laboratory Examinations* Urinalyses, negative. Blood red blood cells 1,800,000, white blood cells 8,100 per c. mm., polymorphonuclears 67 per cent, hemoglobin 71 per cent (Newcomer). Blood Wassermann reaction, negative. Sugar (fasting) 132 mg., urea nitrogen 11 mg. per 100 c.c. Gastric contents showed achlorhydria even after the injection of histamine. Sedimentation rate, 34 mm. on admission and 55 mm. in August, at the end of one hour, corrected for cell volume. Fluid aspirated from the right knee joint on May 10 showed 2,000 cells per c. mm., specific gravity 1.026, Rivalta test, positive. Smear of sediment showed the cells to be mostly polymorphonuclears, but no bacteria either on direct smear or culture were found. Respiratory quotients, after the high carbohydrate diet, 0.89 (average of six determinations), basal calories per square meter per hour, 31 (average of several determinations).

*Course* Upon admission a diet of 500 gm of carbohydrate, 60 gm of protein, and 50 gm of fat was prescribed. She ingested her full allowance of carbohydrate but did not take all of the protein and fat. Nevertheless, her daily food consumption was always in excess of 2,600 calories. Gentle massage and ultraviolet light therapy were given three times a week. The patient took her diet so well that insulin was not used. Her increase in body weight was steady, 13 pounds in twenty weeks. There likewise appeared to be a steady reduction of joint pain and swelling. Upon discharge there was distinct improvement in the range of motion of most of the involved joints.

CASE 6.—*History*: N. B., a woman aged twenty-one years, admitted to the hospital Nov. 2, 1934, and discharged April 19, 1935. She dated her illness to October, 1932, when she noticed a loss of appetite and weight with increasing fatigue after work as a clerk. In March, 1933, pain and reduced motion were observed in the right shoulder. This process spread rather rapidly to the other joints of the upper extremities in spite of a tonsillectomy which was done in May, 1933. Later, following an attack of pharyngitis, the knees became involved. These were placed in casts for three months. The ankles were the last joints to



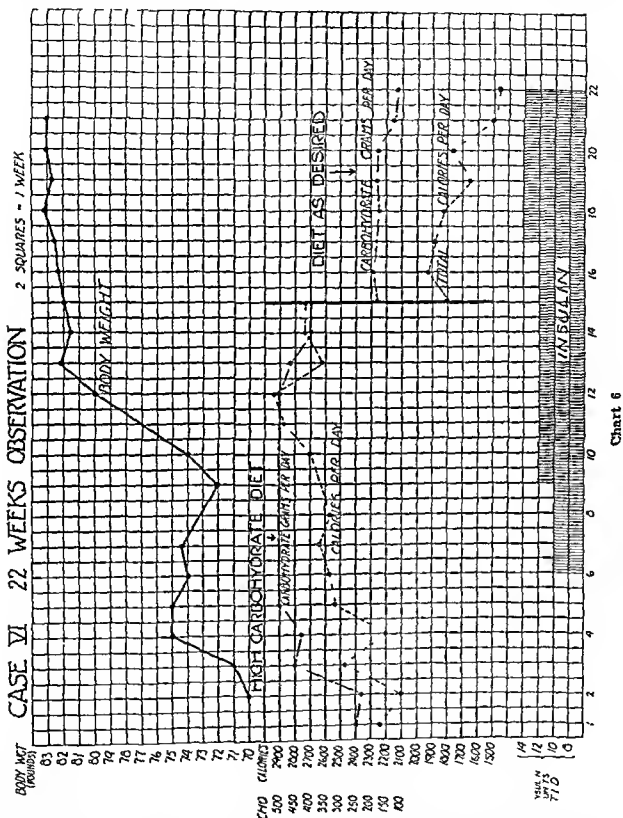
be affected. The total weight loss since the inception of her illness was 30 pounds.

*Examination*: An undernourished young woman who was confined to her bed because of a crippling atrophic arthritis. There was generalized moderate lymphadenopathy. There was obvious infection about one tooth (this was later removed).

*Laboratory Examinations*: Urinalyses, negative. Blood: red blood cells 4,050,000; white blood cells 10,300 per c. mm.; polymorphonuclears 68 per cent; hemoglobin 86 per cent (Sahli). Wassermann reaction, negative. Sugar (fasting) 104 mg.; urea nitrogen 12

mg per 100 cc Sugar taken during digestion while receiving a high carbohydrate diet and 10 units of insulin a c was 1.4 mg per 100 cc Sedimentation rate, 39 mm at the end of one hour, corrected for cell volume Agglutination of *Streptococcus hemolyticus* (AB<sub>13</sub>), positive up to a dilution of 1:2560 of serum Basal metabolic rate, minus 0.2 per cent

Course As is indicated in Chart 6 the patient received a moderate fat and carbohydrate diet for the first two weeks Then the diet was changed to 500 gm of carbohydrate, 50



gm of protein, and 50 gm of fat She also received ultraviolet light treatments and gentle massage three times a week Ferrie ammonium citrate, 2 gm, was given thrice daily Cod liver oil, 8 cc, daily *Streptococcus vaccine* (AB<sub>13</sub>) was given weekly up to doses of 1 cc subcutaneously

After she had been receiving the high carbohydrate diet for four weeks her weight had increased 5 pounds Then insulin was started, 10 units before meals and gradually increased to 14 units She did not gain satisfactorily even though her diet seemed adequate

At the end of the tenth week the protein and fat content of her diet was increased. By the end of the fourteenth week her weight had increased 11.5 pounds. It had been difficult all along to get this patient to eat. Finally she rebelled. As a compromise we offered to let her make out her own menus and have what she wished. This she did for the next six weeks. The result as indicated on Chart 6 demonstrates the difficulties one encounters in getting such patients to voluntarily eat an adequate diet in spite of, in this case, the supposed appetizing effect of insulin. Her weight increased but 1.5 pounds during this period.

She did not improve as some of our other patients, but had on the whole less pain and swelling of the joints. For a time she seemed encouraged and in better spirits. During the

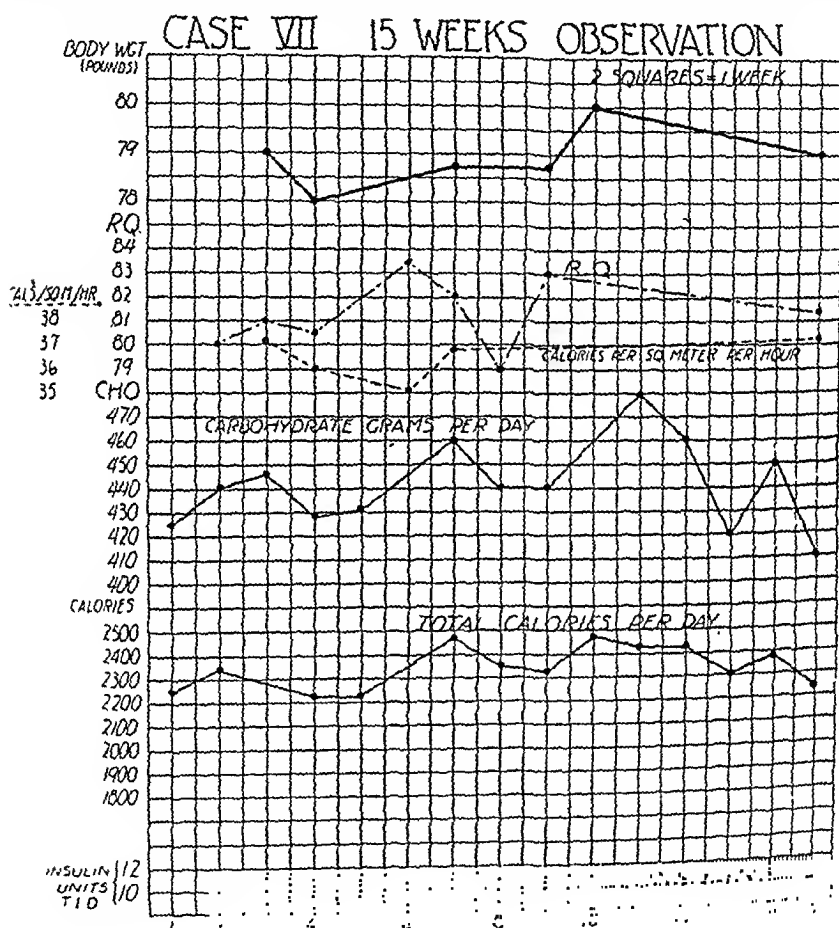


Chart 7.

last two months of her hospital stay she was up occasionally on crutches but never walked any distance. There were days at a time when it was impossible to get her out of bed.

**CASE 7.—History:** M. B., housewife aged thirty-nine, was admitted to hospital on March 2, 1934, and was discharged June 15, 1934. She had a most extensive and advanced atrophic arthritis which involved all the joints of the body including the back. The illness had begun in 1924 following an appendectomy. It had gradually become universal. She had not walked even with the aid of crutches since December, 1933. She had been in many hospitals and had had various types of treatment. Her weight prior to the onset of the disease was 120 pounds; on entrance she weighed 79 pounds.

**Examination:** A completely crippled, emaciated woman who had extreme deformity of all

the joints of the extremities except the fingers and toes. Mouth could be opened about one inch. The patient had to be fed. The legs were contracted on the thighs.

**Laboratory Examinations** Urinalysis, negative. Blood red blood cells 4,500,000, white blood cells 6,200 per c mm, polymorphonuclears 75 per cent, hemoglobin 80 per cent (Tallqvist). Wassermann reaction, negative. Sugar (fasting) 107 mg, urea nitrogen 11 mg per 100 cc. Sedimentation rate 79 mm at the end of one hour, corrected for cell volume. Agglutination of *Streptococcus hemolyticus*, positive up to a dilution of 1:2080 of serum. Gastric contents showed the presence of free hydrochloric acid. Respiratory quotient while

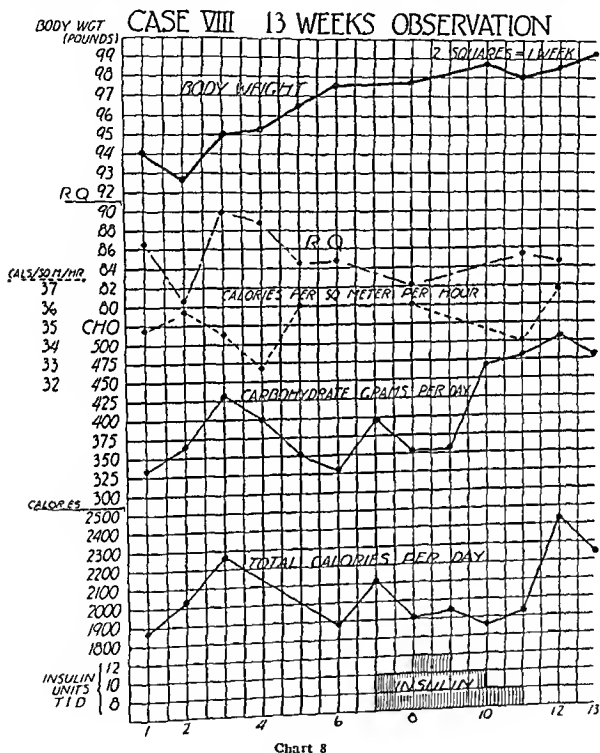


Chart 8

taking a high carbohydrate diet varied between 0.80 and 0.86. Basal calories per square meter per hour varied between 34.7 and 37.7 (six determinations).

**Course** On admission she was given a diet of 150 gm of carbohydrate, 60 gm of protein, and 60 gm of fat. Considering her badly nourished state, she ate remarkably well, as indicated in Chart 7. Her daily consumption of food ranged between 2,250 and 2,470 calories. She usually took most of the carbohydrate allowed. At the tenth week, her weight having remained at 79 pounds, insulin was started, 12 units before meals. This was continued for five weeks without there having been any increase in food ingested or increase in weight.

She had slight fever, 100° F., occasionally. This was unexplained. On the whole, as would be expected, there was no essential change in her state, although she did have less pain and the joints of the upper extremities did appear to have some increased range of motion.

**CASE 8.—History:** D. W., a woman aged fifty-four, was admitted to the hospital on Aug. 25, 1933, and was discharged Jan. 3, 1934. She had had severe attacks of joint pains since May, 1932. There had been recurrent swelling and redness of the involved joints but these never subsided entirely. The fingers of both hands, left wrist, right shoulder, and right elbow were principally involved. The knees and ankles were affected but not so extensively. It appeared from her "past illness" that she had had fleeting, mild joint pains following a respiratory infection in 1928. Also, in the same year urinary symptoms, frequency, urgency, and burning, had been present. She had lost 25 pounds in the past year. Twenty-three years ago she had probably had an osteomyelitis of the coecyx which was opened twice. Her teeth had all been removed two years previously because of pyorrhea.

**Examination:** The patient was an undernourished woman with atrophic arthritis involving the joints as stated in the history. The knees and shoulders were normal objectively but gave pain on motion. The ankles were swollen and painful on pressure. There were a few rales in the right subscapular region with bronchovesicular breathing. There was a tender mass in the upper right quadrant, probably kidney.

**Laboratory Examinations:** Urinalyses all showed a trace of albumin, no sugar, and the sediment always showed many leucocytes. Blood: red blood cells 4,600,000; white blood cells 5,600 per c. mm.; 71 per cent polymorphonuclears; hemoglobin 75 per cent (Sahli). Wassermann blood reaction, negative. Sugar (fasting) 105 mg.; urea nitrogen 11 mg.; calcium 9.5 mg.; phosphorus 3.5 mg.; uric acid 2.5 mg. per 100 c.c. Respiratory quotient, varied from 0.81 to 0.89 (six determinations). Basal calories per square meter per hour, varied from 32.7 to 37.1.

**Course:** On Sept. 2, 1933, she was given a high carbohydrate diet as indicated on Chart 8. She did not eat well, usually less than 2,000 calories. She had, however, a urinary tract infection and frequently had slight fever. The bladder urine showed a few leucocytes with many small gram-negative bacilli. The injection of sterile water into the right renal pelvis reproduced the pain which the patient had experienced in the upper right quadrant. The ureteral urine, however, was clear. It was thought unwise to do a retrograde pyelogram. X-ray of the kidneys after the intravenous injection of skiodan showed the right kidney pelvis to be definitely larger.

Her weight remained between 93 and 95 pounds for the first six weeks. Then she was given insulin, 10 units before meals. After this her average food intake was somewhat greater. At the end of the thirteenth week her weight was 100 pounds.

There was no essential change in her general state. At the time of her discharge she could use her hands very much better.

**The Factor of Undernutrition in Chronic Atrophic Arthritis.**—The majority of patients with this type of arthritis lose weight, especially when the joint lesions become multiple, and the loss of weight is usually commensurate with the severity of the arthritis. In our perusal of many textbooks and key articles on this subject, no accurate data on the loss of weight was found. Since it seemed important to know whether the patients that we had chosen for this study were representative of advanced arthritis in that stage, the weight loss of 18 similar patients in addition to those reported was determined. The maximum loss was 48 pounds, the minimum, 15 pounds, and the average, 28 pounds.

Others have attempted to increase the weights of individuals who had become poorly nourished during the course of arthritis. Howitt and Christie<sup>4</sup> endeavored to increase the weight of twenty patients with chronic atrophic arthritis. These were observed both in the hospital and as out-patients. They were given a diet which, in the opinion of these workers, exceeded the patients' requirements

TABLE I  
SUMMARY OF DATA

CASE	AGE (YEARS)	PERIOD OF OBSERVATION (WEEKS)	WEIGHT LOSS BEFORE ADMISSION (POUNDS)	WEIGHT GAIN (POUNDS)	INSULIN PERIOD (WEEKS)	AVERAGE F Q	AVERAGE B M R (PER CENT)	CLINICAL CHANGES
1	52	43	31	12	6	0.83	-12	1 from bed to ambulatory Less pain Greater range of joint motion
2	66	65	40	50	11	0.90	-3	Much less pain, greater range of joint motion Could have walked if legs could be extended
3	72	31	31	24	254	0.83	+7	Great improvement Knees and hip joints much increased range of motion
4	18	33	50	11	3	0.83	+1	No change in arthritis, pulmonary tuberculosis
5	11	20	40	13	0	0.91	-10	Considerably less pain and increased range of joint motion
6	21	22	30	13	16	---	---	Slight, but definite improvement
7	39	15	41	0	6	0.82	+2	No change in arthritis
8	51	13	23	7	1	0.85	+0.7	Slight improvement in joint symptoms



by 500 to 1,000 calories. Insulin in doses of 10 to 15 units twice daily was given for a period of six weeks. Some weight was gained in each case; the maximum was 10 pounds. The general health and well-being of all the patients was improved. Copeman<sup>2</sup> reported measurable improvement in three patients who had been given a pound of glucose daily in addition to a normal diet. During this period of observation, three to four weeks, the patients received increasing amounts of insulin up to 60 units daily. The weight gains were only 3 to 5 pounds. Eaton and Love<sup>3</sup> treated 22 undernourished arthritic patients with a high caloric diet and insulin up to 90 units daily for short periods. The average weight increase was 12 pounds in four to seven weeks. With these gains the authors state that there was noticeable improvement in the arthritis. Dawson<sup>4</sup> reported that he had given diets high in carbohydrate to a number of patients with rheumatoid arthritis and that rather marked improvement had followed.

#### SUMMARY AND CONCLUSIONS

Eight patients with advanced atrophic arthritis, all women, whose ages varied from eighteen to sixty-six years, were observed in the hospital, during which time they were fed a high carbohydrate diet. The shortest period of observation was fifteen weeks, while the longest was sixty-five weeks. All patients had lost weight excessively, the maximum being 50 pounds and the minimum, 25 pounds.

Seven of the patients received insulin for the purpose of observing its action on increasing appetite; also, the influence of the reduction of blood sugar on the arthritis. All patients were observed for varying periods on the diet alone before insulin was administered. During this fore-period every effort was made by all the hospital services to induce the patients to eat. Carbohydrate was given to them in many forms. After this preliminary study further nutrition was attempted by the use of insulin up to the point of the patient's tolerance. It will be observed by examination of the charts that the weight curve was not conspicuously steeper during the period of insulin administration. Nor was the amount of food ingested greatly increased. Following the sudden withdrawal of insulin, salt solution subcutaneously was substituted; usually this was not accompanied by any diminution of appetite.

Three patients, Cases 1, 2, and 3, those who had been under observation for the longest periods, made the greatest weight gains, 42, 50, and 24 pounds, respectively. They also made the greatest clinical improvement. The texture of the skin of the patients who gained weight also was noticeably changed from the thin, atrophic skin, which is so commonly seen in such patients, to one of a more normal elasticity and firmness. Two patients, Cases 4 and 7, even though they did ingest adequate calories, failed to gain; both had the maximum devastation of the disease. One patient, Case 5, who had gained a lesser amount, also improved remarkably.

Clinical improvement that was observed in these patients cannot be ascribed to a single measure because several factors were obviously operating—rest, freedom from worry, and exposure, and a large amount of vitamin C. As far as possible other adjuncts were employed in a minimum degree. The high carbohydrate diet did not produce any exacerbation of the arthritic process. Only

one patient. Case 3, developed arthritis in a new joint: this was noticed soon after admission. There was no increase in symptoms either objectively or subjectively.

The respiratory metabolism was studied in seven of the cases. In six it was followed throughout the period of observation at frequent intervals. We anticipated that the initial metabolic rates would be reduced as a result of their undernourished state. Only one patient (Case 1) did have a temporary slight lowering of the rate early in the study, which was possibly due to an antecedent respiratory infection. Also, these rates were found to be steady throughout the period of observation and to be unaltered by increasing body weight.

The trend of the postabsorptive respiratory quotients, even though they showed in some cases rather wild variations, was approximately level throughout. The quotients during the periods of insulin administration were not altered. The average quotient was found to be slightly higher than that of individuals who eat the ordinary mixed American diet.

It then seems that the seven patients with advanced chronic atrophic arthritis, upon whom postabsorptive respiratory quotients were determined while they were taking a high carbohydrate diet, are able to use carbohydrate normally.

We do not, however, believe that a high carbohydrate diet has any special efficacy in the treatment of chronic atrophic arthritis, but we do stress the importance of overnutrition in the management of such patients when they are undernourished.

This study would not have been possible without the untiring cooperation of the hospital dietitian, Miss Dorothy Love, her assistant, Miss Evelyn Vogel, and the laboratory assistants, Miss Grace E. Sly and Miss Rachel W. Lee.

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#### DISCUSSION

DR. CHARLES W. SCULL, ELLEN'S PARK, PA.—Drs. Bowen and Lockie have added significant data to the chapter on the dynamic pathology of arthritis in reference to carbohydrate metabolism. The conclusion that the undernourished arthritic patient uses carbohydrate normally may at first thought appear to be inconsistent with the fact that many arthritic patients show a delayed rate of removal of glucose when subjected to a tolerance test. However, the "delayable" response of the arthritic patient may be referable to circulatory disturbances rather than to an inherent deficiency of carbohydrate metabolism. The present data indicating an essentially normal respiratory quotient and a negative effect of insulin upon the nutrition of the arthritic patient lend further probability to this view.

The present observations also form a basis upon which the problem of nutrition should progress with particular regard to caloric balance and the qualitative composition of the diet. Drs. Bowen and Lockie studied severely undernourished patients. Measures to correct this state of relative starvation are clearly indicated. One of the first requisites toward this end is a caloric supply in excess of the energy output. The desideratum, however, is not merely the storage of calories in the form of fat, but the achievement of a

state of optimal nutrition. The problem of restoring nutritive balance in a malnourished arthritic patient is not a simple matter of providing an abundant supply of calories in the form of any one foodstuff. The correction of gross malnutrition involves as many factors as are involved in the growth process. Either absolute or relative deficiencies of any of the dietary components which are not interchangeable with one another may constitute limiting factors to normal growth. If insufficient iron is supplied, hemoglobin cannot be produced; if calcium or phosphorus be lacking, normal ossification does not occur; if iodine is lacking, thyroxin cannot be produced; if essential amino acids are inadequate in amount, the protein structural units of tissue, viz., muscle, the organic matrix of bone, tendons and cartilage cannot be elaborated. A net loss of these essential factors from tissues in disease leads to structural collapse. On these premises it is apparent that a high carbohydrate supply alone cannot be expected to correct malnutrition, to compensate for amino acid deficiencies or replace inorganic salts, essential fatty acids, or accessory food factors.

These considerations are of more than academic importance. Drs. Bowen and Lockie have recorded the fact that three of the eight patients even while receiving an adequate supply of calories in the diet failed to make striking progress so far as the arthritis and nutritional state were concerned. While in them factors other than dietary may have prevented progress, attention may be called to the fact that some selected cases on the high carbohydrate, high caloric diet equilibrated with respect to bed rest, removal of foci, etc., may experience a marked benefit when the diet is changed to one richer in protein, fat, vitamins, and salts. Several explanations have been advanced to account for this effect. It has been noted in our laboratory and by Adlersberg and Forges in Germany that such dietetic changes are accompanied by a loss of fluid from the body along with a reduction of tissue swelling. Pevsner and other Russian investigators have noted that such a dietetic alteration is accompanied by a reduced sensitivity of the arthritic patient in an allergic sense.

Irrespective of the mechanism whereby such dietetic differences become effective they emphasize the fact that some practical approximation of a dietary prescription may be made which provides not merely a tolerable, not merely an adequate, but an optimally constituted mixture out of which the body may maintain, repair and build its tissues and function at a level approaching optimal physiology. Some arthritic patients may get well in the presence of infected teeth, or tonsils; some may improve in spite of the handicap of suboptimal diets, but more will get well more rapidly if given the benefit of judicious removal of foci and the application of dietetic measures.

DR. T. PRESTON WHITE, CHARLOTTE, N. C.—Dr. Bowen has used for observation eight cases of atrophic arthritis of some years' duration. As was to be expected, the improvement shown depended on the activity and stage of the disease; inflamed swollen joints are capable of great improvement, whereas, stiff, cold, burned-out joints can hardly be benefited. The observation that the patients gained weight no faster on insulin than without it, is interesting. The effect of the high carbohydrate, low protein and fat diet on articular pathology can only be said to be a negative one. Only one new joint became involved and the old ones did not become worse.

The improvement noted in about one-half of his cases may have been due in some measure to the greater supply or the greater utilization of carbohydrates, but it is just as reasonable to attribute improvement to the forced eating of the remainder of the diet, to prolonged bed rest, or to proper elimination.

The rôle of diet in arthritis is still unknown. The difficulty lies in controlling the many other factors while the effects of various diets are being studied. In our experience patients suffering with atrophic arthritis do best when treated as a whole, placing them on a balanced diet that would restore the weight to a normal level, giving the patient complete rest, and paying the closest attention to the question of bowel elimination. Physical therapy is a most helpful aid.

The improvement obtained by our patients seems to be directly in proportion to the amount of rest and to the careful attention to elimination. When the disease has become arrested, patients eat what they want. Those patients who have stayed well over a long period of time have told me that rest and elimination are the most important factors from their standpoint. In my part of the country most people are big bread eaters and because

of the quantity of bread consumed they do not eat enough vegetables and fruits. Most of these people are definitely benefited by curtailing their carbohydrate intake and insisting on more fruits, vegetables, and red meats.

We may well take a leaf from the book dealing with the treatment of tuberculosis, and in the beginning, stress to the patient suffering with arthritis the importance of the length of time which it takes to arrest it, and, when it becomes arrested, that he must lead a life which does not permit exhaustion and that he will never be able to go at the same pace as those people who have not been affected by the disease.

DR PHILLIP S HENCH, ROCHESTER, MINN.—Drs. Bowen and Lockie are not proposing a new diet for the much dieted arthritic patient. Admitting that there is no special efficacy to a high carbohydrate diet for atrophic arthritis they have, however, shown that such a diet is apparently quite harmless. One is permitted to conclude that they believe that restrictions in carbohydrate intake have no particular merit either.

When one studies patients with atrophic arthritis intensively, one finds physiologic aberrations pertaining not only to carbohydrates but also to sulphur, cholesterol, perhaps to calcium, and other substances. The apparent disturbances in carbohydrate metabolism, which, by the way, are by no means consistently present, have not seemed to me to be of greater significance than other alterations and the rationale for a low carbohydrate diet in the treatment of atrophic arthritis has not been proved to my satisfaction. Nevertheless, when such a diet was proposed again several years ago we gave it a trial in about two hundred cases with, in general, disappointing results. Very few patients whose relief is ascribed to a low carbohydrate diet are actually treated by that means alone. Most of them have also had some infected focus removed, are given physiotherapy, and certain extra amounts of rest in a hospital or at home.

At the Mayo Clinic, we have abandoned the use of a low carbohydrate diet as a routine measure although we are quite willing to prescribe it to a patient who insists that carbohydrates make the joints worse. However one often finds on quizzing such a patient, that she never noted a relationship until she read in a health column, or was told by her physician that she should avoid carbohydrates. When later she ate some, it was perhaps her conscience more than her joints that hurt. A shifting barometer may unwittingly have provided increased pain coincident with her dietary "indiscretion." At any rate, we have found the stories of such patients very inconsistent. They blame one carbohydrate containing food, not another which contains equal amounts. They note joint pains after eating carbohydrates in one form but not when they eat the same carbohydrate containing food in another form. We have not been able to convince ourselves that any one type of carbohydrate or carbohydrates in normal or even excess amounts, are specifically harmful.

Appropos of the apparent harmlessness of a high carbohydrate intake, you may recall that two years ago, before this conference, I reported on the analgesic effect of jaundice in certain rheumatic diseases. I have now studied about forty patients with atrophic arthritis and fibrositis, whose disease was largely or completely inactivated by various types of jaundice. While these patients were in the hospital under the care of Drs. Snell, Weir, Comfort, and myself they were routinely given 400 to 500 gm. of carbohydrate daily (bread, cereals, potatoes, crackers, jellies, cakes, fruit juices and candy between meals). They were on this diet three to five weeks in the hospital and at least three to six months thereafter. While in the hospital they were generally given also an average of 100 gm. of sugar intravenously, daily for three weeks. Glycosuria was often produced but the analgesia and reduction of stiffness and swelling of joints were in no way disturbed by this intake of 400 to 600 gm. of carbohydrate daily for many weeks.

DR BOWEN (closing).—I neglected to speak of the difficulty we experienced in getting our patients to eat even the rather low protein content of their diets. The amount that they actually ingested was usually 5 to 10 gm. lower in protein than was served them.

# THE PRESENT STATUS OF FEVER THERAPY IN THE TREATMENT OF GONORRHEAL ARTHRITIS, CHRONIC INFECTIOUS (ATROPHIC) ARTHRITIS, AND OTHER FORMS OF "RHEUMATISM"\*

PHILIP S. HENCH, M.D., ROCHESTER, MINN.

VARIOUS methods for the production of fever in "fever therapy" include diathermy; radiothermy: heated, air-conditioned cabinets; hot baths, and heated air currents. Regardless of the method used, certain, and at times rather profound, physiologic effects are produced. These include alterations in the blood flow, in the chemical and cellular elements and immune bodies of the blood, in the content of sweat and gastric secretions, in the amount and reaction of urine, in the metabolic rate, and in electrocardiograms. Some of the reactions are of little consequence either from the standpoint of discomfort or relief. The most important are the effects of therapeutic hyperpyrexia on the growth of invading organisms, such as the bacteriolytic and bacteriostatic effect of fever therapy on gonococci and on the spirochete of syphilis.

Since 1931, fever therapy has been used in the treatment of various "rheumatic diseases," with striking success in the gonorrheal type and with moderate success in other types. Twenty reports on the results of fever therapy in gonorrheal arthritis have been made in this country: ten have been published, and the reports have been summarized by Hench, Sloeumb and Popp,<sup>1</sup> and ten were given at the Fifth Fever Therapy Conference in Dayton in May, 1935.<sup>2, 3</sup> (Table I). Twenty reports on results in acute and chronic infectious (atrophic) arthritis have likewise appeared: sixteen have been published<sup>1</sup> and four were made at the recent conference<sup>2, 3</sup> (Table II). There has been a considerable variety in the methods used, in the dose of fever, and in the number of sessions of fever given. The results have varied considerably in earlier reports, less so recently. An analysis of results does not suggest that one method is superior to another so far as results in the diseases treated are concerned, and a choice of method resolves itself into the selection of that one which is the most comfortable, the least dangerous, and the least expensive to the patient. The majority of workers to date seems to favor the use of heated, air-conditioned cabinets, such as the Kettering hypertherm. The use of such cabinets seems to be less exhausting than hot baths or diathermy hyperpyrexia, and less expensive and less likely to produce cutaneous burns than radiothermy. The oral administration of 3 to 4 liters of 0.6 per cent solution of sodium chloride during the sessions of fever markedly reduces unpleasant reactions.

Gonococci are generally killed by five to seventeen hours of fever at 106.7° F. (41.5° C.); a few hardy strains are killed only after twenty-seven hours of such a fever. Since these amounts of fever can be equalled or exceeded in a single or in divided doses in the treatment of patients, the effect of fever therapy

\*From the Mayo Clinic.

TABLE I  
FEBRILE THERAPY FOR GONORRHEAL ARTHRITIS: REPORTS AT FIFTH FEVER THERAPY CON-  
FERENCE, MAY, 1935

AUTHOR	DOSE OF FEVER RECOMMENDED, HOURS AND OF GRADES F	TOTAL NUMBER SESSIONS OF FEVER	PATIENTS TREATED	RESULTS, PER CENT				AUTHORS' COMMENTS
				SYMPTOM FREE CURED	MARKED RELIEF	MODERATE RELIEF	LITTLE OR NO RELIEF	
Warren, Car- penter and Berk	5 to 17 hr at 106.7°	1, equal to ther- mal death time of pa- tient's strain	15	87	*	*	*	Patient may be infected with two strains, that of patient and con- sort generally about equal Strains from urethra and joints may have different thermal death time (usually a little shorter from joints)
Sackett Burman	*106°	1 to 2+	18 16	61 81		33 (	6 13	Complete cure if bony changes not present. Physiotherapy for residual stiffness
Hefke	5 to 6 hr at 105 to 106°	3	1	100				
Arnall		*	19	89		11		Results remarkable
Epore	*106 to 107°	*	6	100				
Schindler	5 hr at 106 to 107°	2 to 6	Acute 9 Chronic 9	56	100 33	11		In acute cases results "striking" to "marvelous"
Tennant		*	3	100				
Strickler	6 to 7 hr at 105 to 107°	4	Acute 9 Chronic 4	45	22	22	11	No better than other treatment
Kendall and Webb	6 to 7 hr at 106 to 107°	*	Acute 19 Chronic 12	84	*	*	23	
Todt			113	72 per cent	8 per cent	*	*	
						20	per cent	

\*Data incomplete

TABLE II  
 FEVER THERAPY FOR INFECTIOUS ARTHRITIS: REPORTS AT FIFTH FEVER THERAPY CONFERENCE,  
 MAY, 1935

AUTHOR	DOSE OF FEVER RECOMMENDED; HOURS AND DE- GREES F.	TOTAL NUMBER SESSIONS OF FEVER	PATIENTS TREATED	RESULTS, PER CENT				AUTHORS' COMMENTS
				SYMPTOM- FREE; "CURED,"	MARKED RELIEF	MODERATE RELIEF	LITTLE OR NO RELIEF	
Stocker	3 hr. at 103 to 104°	1 to 4	Acute 13		46	31	23	If no relief with first session, treatment discontinued; No better than other treatment; temporary relief of pain Two patients had diabetes; not considered a contraindication
Strickler	6 to 7 hr. at 105 to 107°	4	Chronic 33	25	33	27	40	
Hofke	*103 to 105°	4 to 5	Chronic 31	7	50	58	25	
Tenney	3 to 4 hr. at 104 to 106°	*	Early 10		60	20	35	
			Late 13		23	23	54	
Total			129	About 7 per cent	About 30 per cent	"88 per cent improved,"	14	

\*Data incomplete.

in gonorrheal arthritis is apparently to sterilize the joints through a direct bacteriolytic effect. The usual plan of treatment is to give two to six sessions of fever at  $106^{\circ}$  to  $107^{\circ}$  F, for five to six hours. Recently, Warren, Carpenter, and Boak have proposed an alternative plan in cases in which the thermal death time of the infecting strain of gonococci can be determined—one prolonged session (five to seventeen hours) is given which is equivalent to the thermal death time (at  $106^{\circ}$  to  $107^{\circ}$  F) of the patient's particular strain. This method may be less expensive and is of course less time consuming, it may not, however, be practical for other than selected cases.

#### RESULTS IN ACUTE GONORRHEAL ARTHRITIS

In spite of growing conservatism in estimating the results, those obtained in gonorrheal arthritis are striking. In acute gonorrheal arthritis the results have been as follows (figures are given in round numbers and are therefore approximate, percentages for those receiving less than notable or marked relief are omitted here). Of the first 24 patients treated (reports published earlier, from various sources), 90 per cent were promptly cured and 10 per cent received little or no relief. Of 118 cases discussed at the recent conference, 80 per cent of the patients became symptom free, 10 per cent were markedly relieved, and 10 per cent but slightly relieved. Of 9 patients treated at the Mayo Clinic, 5 were promptly cured, the rest markedly relieved. Thus, of a total of 151 patients with acute gonorrheal arthritis, treated by a number of different physicians in various parts of the country, 80 per cent have apparently been cured and an additional 10 per cent have been markedly relieved (Table III).

#### RESULTS IN CHRONIC GONORRHEAL ARTHRITIS

The results in chronic gonorrheal arthritis (of more than six weeks' duration), while very good, are less striking (Table III). In 25 cases reported at the conference, 40 per cent of patients were "cured," 30 per cent markedly relieved. In 7 from the Mayo Clinic, 25 per cent were cured, 45 per cent markedly relieved. Thus of a total of 32 patients, 35 per cent were cured and 30 per cent were markedly benefited.

#### RESULTS IN ACUTE INFECTIOUS (ATROPHIC) ARTHRITIS

Doses of fever available in fever therapy are inadequate to kill the streptococci presumably responsible for acute and chronic infectious (atrophic) arthritis. Such results as are obtained arise presumably from possible bacteriostasis, augmentation of the patient's resistance, and vasodilatation. The usual plan is to give three to eight sessions of fever at  $104^{\circ}$  to  $105^{\circ}$  F for about four to five hours. Of 21 patients with acute (nonspecific) infectious arthritis whose cases were reported at the conference, 10 per cent only were "cured" but an additional 40 per cent were markedly relieved (Table III).

#### RESULTS IN CHRONIC INFECTIOUS (ATROPHIC) ARTHRITIS

Of a total of 147 patients with chronic infectious arthritis previously reported on by a number of physicians, 10 per cent became symptom free, 25 per



cent were notably benefited. Of 108 patients reported on at the conference by several workers only 5 per cent became symptom-free but an additional 30 per cent were notably relieved. At the Mayo Clinic, 60 patients have been treated;

TABLE III

FEVER THERAPY--SUMMARY OF RESULTS TO DATE (PERCENTAGES ARE GIVEN IN ROUND NUMBERS AND ARE THEREFORE APPROXIMATE)

DISEASE	PATIENTS TREATED	RESULTS, PER CENT			
		SYMPTOM-FREE; "CURED"	MARKED RELIEF	MODERATE RELIEF	LITTLE OR NO RELIEF
Gonorrheal arthritis, acute	Published	24	90		10
	Conference	118	80	10	10
	Mayo Clinic	9	50		
	Total	151	80	10	
Chronic (more than 6 weeks)	Published, undifferentiated from above				
	Conference	25	40	30	30
	Mayo Clinic	7	25	45	15
	Total	32	35	30	
"Infectious arthritis" (atrophic) acute	Conference	21	10	40	50
Chronic	Published	147	10	25	35
	Conference	108	5	30	65
	Mayo Clinic	60	0	20	20
	Total	315	5	25	
Senescent arthritis (hypertrophic)	Published	74	5	50	45
Gouty arthritis (chronic)	Published	1	100		
Traumatic arthritis (chronic)	Published	2	100		
Neuritis	Published	6	80	20	
Myositis	Published	8	25	75	
Bursitis	Published	4	50	50	

none was cured, 20 per cent considered themselves, and were considered, to be markedly benefited. Thus of a total of 315 patients treated in different clinics, 5 per cent became symptom-free and 25 per cent were markedly relieved. The remainder received little or no benefit (Table III).

A very few patients with senescent (hypertrophic), chronic gouty, and chronic traumatic arthritis, as well as a few with neuritis, myositis, and bursitis, have been so treated. Those with senescent arthritis have not been particularly benefited except in a few instances. From 25 to 50 per cent of those with the other types of arthritis have been reported as notably helped (Table III).

#### CONCLUSION

From the results collected from various parts of the country to date, one may tentatively conclude that, if early and adequate treatment is given to a patient with gonorrheal arthritis, he has an 80 per cent chance of being promptly cured, and if not cured an additional 10 per cent chance of being markedly relieved. The treatment is almost specific, and it is almost completely successful when bony changes have not occurred. A patient whose gonorrheal arthritis is

of more than six weeks' duration has lost valuable time. Even so, such a patient has a 35 per cent chance of being more or less promptly cured, and if not cured, then a 30 per cent chance of being markedly relieved. Physiotherapy may be needed for residual stiffness. Ankylosis may persist. In any event, fever therapy is the method of choice and in any case of acute gonorrheal arthritis it should be promptly instituted.

In infectious (atrophic) arthritis, results are often gratifying occasionally striking, but in general disappointing. The patient with acute infectious (atrophic) arthritis has a 10 per cent chance of becoming symptom free, a 40 per cent chance of receiving notable relief. The patient with chronic infectious (atrophic) arthritis has only a 5 per cent chance of becoming symptom free, a 25 per cent chance of being notably helped. A welcome remission may be induced. A "cure" is not to be expected. The best results are obtained when the disease is of less than a year's duration. Further experience is necessary to determine the exact value of this treatment in chronic infectious arthritis. It can hardly be considered successful in cases in which patients receive only moderate relief, for such relief can be obtained almost routinely by less expensive and less strenuous methods of treatment. For patients inadequately relieved by other measures, or who are intolerant of slower methods a trial of fever therapy seems justified.

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#### DISCUSSION

DR. W. J. STAINSBY, NEW YORK, N. Y.—Every year for years several forms of therapy have been advocated with enthusiasm for the cure of either gonococcal or more particularly, atrophic arthritis. With few exceptions after the period of enthusiasm has subsided these new forms of therapy have been discarded. One of these exceptions is the removal of foci of infection. Many years ago Billings pointed out the relation of foci of infection to arthritis. After a period of enthusiasm in which many innocent teeth and tonsils were removed, the value of this form of therapy took its proper place. At the present time we recognize it as being of distinct value in a limited group of patients with arthritis. However, as far as the large majority of patients is concerned, this form of therapy is not of much use. More recently we have had a wave of enthusiasm over vaccines. Various forms of vaccines were used for a period of about ten years. After the wave of enthusiasm subsided many arthritic specialists were not so enthusiastic about the improvement, and no definite evidence is at hand that vaccines definitely modify the course of the disease. That applies to gonorrheal arthritis as well as atrophic arthritis. I am speaking of permanent cures, not temporary relief. There are of course many measures that temporarily relieve the symptoms. With this background you can see why I view with some skepticism the recent wave of enthusiasm about fever therapy.

Dr. Hench's results correspond with the reports of other investigators with this form of therapy in gonococcus arthritis. I would like to consider all these reports as preliminary and wait for more definite evidence of the value of this form of therapy in gonococcus arthritis.

Dr. Hench's results in atrophic arthritis are much less striking. They correspond with our own. In 1933 Nicholls, Hansson, and I reported 12 patients treated with this form of

therapy. In all cases there was temporary relief of the symptoms, but at no time did we find that this form of therapy cured the disease.

In my opinion this form of therapy in gonorrheal arthritis is promising at the present time, but is of no use in the treatment of atrophic arthritis.

DR. WALTER BAUER, BOSTON, MASS.—Dr. Charles L. Short and I began the treatment of patients with arthritis by means of hyperpyrexia the latter part of 1930. We wish to reemphasize what Dr. Hench has said concerning the value of this treatment in gonorrheal arthritis. It is the nearest thing to a specific therapy that has ever been employed for this type of arthritis. Our certainty regarding the value of this form of therapy in gonorrheal arthritis has been based on the thorough studies of Dr. Stafford Warren and his group at Rochester. Without their detailed bacteriologic studies on the thermal death point of various strains of gonococci, we would be unable to prescribe treatment intelligently or properly to interpret the results.

The results obtained in treating atrophic arthritis with hyperpyrexia are not very encouraging when compared with those obtained in gonorrheal arthritis. We have treated a total of 25 cases of atrophic arthritis with a total of 71 treatments. Following one or more treatments by means of a Victor superpower diathermy machine and electrodes, the patients were observed for a period of one to three years or more. The number of treatments given each patient varied from one to fifteen and the usual temperature maintained was 104° for four hours.

In 20 out of our 25 cases, temporary improvement resulted both subjectively and objectively. The results were at times almost miraculous, but unfortunately of very short duration. In only 7, or 20 per cent, was this gain maintained to the end of the follow-up period. The end-results are best shown in table form.

DURATION OF IMPROVEMENT

DURATION	CASES
None	5
Up to 2 weeks	8
From 2 weeks to 6 months	6
From 6 months to 1 year	1
From 1 year to 2 years	2
Two years or more	3

Of the 5 patients who improved, one became pregnant within a month after her last treatment and has continued free of pain. It is known that pregnancy may induce a remission in atrophic arthritis. In another patient, a remission apparently had started two months before the treatments were begun. A third patient has pursued an unusually faithful course of rest and physical therapy at home. Therefore, in three of the five patients who received benefit, it is not possible to ascribe the entire improvement to diathermy hyperpyrexia. Those who improved were younger, their arthritis of shorter duration with little or no cartilage destruction and two had marked vasomotor symptoms.

Therefore, balancing the results obtained against the severity of the treatment, our conclusion is that in atrophic arthritis, the use of this method is only occasionally justified and should not be used to the exclusion of general treatment.

DR. ROBERT B. OSGOOD, BOSTON, MASS.—I believe it is the informed and wise opinion of the people who have tried it, as Dr. Hench and Dr. Bauer well know, that this form of treatment should only be administered in a well-equipped hospital with attendants who are trained in its use. If this method were generally used, I think we should expect some disastrous results.

DR. R. GARFIELD SNYDER, New York, N. Y.—May I reemphasize Dr. Osgood's warning? I think fever therapy is a very dangerous form of treatment, and should be used only in heroic cases when everything else has failed to bring about improvement. I have had

one case of gonorrheal arthritis in which this method of treatment was used with a fatal outcome. The treatment was carried out at the hospital and the patient's temperature was elevated to 104° F. Soon after completion of the treatment the patient's temperature rose to 106° F. then to 107° and death followed. There seemed to be no explanation for what occurred, except that the rise of temperature induced by the fever treatment continued progressively through some unexplainable loss of control in the temperature controlling center of the brain.

WALTER M. SIMPSON, DAYTON, OHIO—High, sustained, controlled artificial fever is the treatment of choice for gonorrheal arthritis. Gonorrheal arthritis is a manifestation of a systemic disease, requiring systemic treatment. *In vitro* thermal death time studies, and the clinical response of patients with gonococcal infections to artificial fever therapy, indicate that it is possible in most instances to destroy gonococci in the various lesions of the disease with high, sustained body temperature. In addition to this sterilizing effect, there is evidence that artificial fever therapy stimulates immune reactions.

Thirty one patients with gonorrheal arthritis associated with gonococcal infection of the genitourinary tract, have been treated by us with artificial fever therapy utilizing a simplified, relatively safe and controllable air conditioned apparatus, known as the Lettering hypertherm. Of 19 patients with acute gonorrheal arthritis the average improvement in joint function immediately after the conclusion of the course of fever therapy was 75 per cent, in three patients the restoration of joint function was complete. The ultimate average improvement in joint function in the cases of acute gonorrheal arthritis was almost 100 per cent, 13 patients have obtained complete restoration of joint function. Of the 12 patients with chronic gonorrheal arthritis the average improvement in joint function at the conclusion of the course of fever therapy was about 60 per cent, in 4 patients joint function was completely restored. The ultimate improvement in joint function in cases of chronic gonorrheal arthritis was almost 90 per cent. At the conclusion of the course of fever therapy gonococci had disappeared from the smears of the genitourinary tract of 24 patients. The urethral smears of 4 patients became negative within a week following the conclusion of the fever treatments. Supplemental treatment eliminated all evidence of gonococcal infection of the genitourinary tract of the remaining 3 patients.

In 2 cases of chronic gonorrheal arthritis almost complete limitation of motion of one knee joint remained after the conclusion of the course of artificial fever therapy. Orthopedic manipulation (*brisement forcé*) under general anesthesia was done to separate fibrous adhesions. Artificial fever therapy was reinstituted immediately following the surgical manipulation. Practically normal joint function has been restored in both cases.

## WHAT CAN BE EXPECTED FROM THE ORTHOPEDIC CARE OF ARTHRITIS?

LORING T. SWAIM, M.D., BOSTON, MASS.

I AM not going to discuss the treatment of arthritis except to try to bring to your attention what can be expected from orthopedic care in chronic arthritis. The word orthopedic means "straight child." Nowhere in the practice of orthopedic surgery is the name more truly exemplified than in chronic arthritis because it is so vitally important that every patient with chronic arthritis should be properly balanced mechanically after he is well. Many patients with arthritis have been cured of the active disease but have been so crippled that they could do nothing. They might just as well have not got well. There must be complete understanding between the medical and the orthopedic physicians in every case of arthritis. In order to have successful results in arthritis, each physician must know exactly the plan of the other and what can be expected from cooperative treatment. What can the medical man expect from his orthopedic confrere?

He can expect the prevention of the flexion deformities, if early protective splinting can be started before the deformities have occurred. This means consultation at the very beginning of the arthritis. It means careful observation of the joints at frequent periods to size up the tendencies in each joint, and anticipate the probable deformities which are likely to occur or are occurring. Joint deformities begin very early during the inflammatory period of rheumatoid (atrophic) arthritis. This must be appreciated by whoever is in charge of the case; otherwise the pain with resulting muscle spasm and flexion will insidiously produce deformity. At first this does not seem of great significance. It is at this time that the deformity can be prevented most easily. Complete rest of the joint for a period of a few days to a week is often sufficient to cause a complete subsidence of the acute inflammatory reactions. Pain, so often due to slight, continued trauma of motion, can be entirely eliminated by complete rest in a plaster cast. It is difficult for us to realize how much trauma actually takes part in the production of inflammation in chronic arthritis. I firmly believe that at least 50 per cent of the inflammation in joints is kept up by use during the acute stages of the disease rather than by the actual etiologic factor, whatever it is. I also believe that a great deal of the capsular thickening and the extension of pannus between the surfaces of the joint would be less if trauma were prevented. This has been our experience in the protection of joints in the last eight years. Constant attention to details, with the use of plaster casts and shells for rest, we have found, has lessened danger of ankylosis and stiffening of the joints more than where the joints are not protected and rested. Exercise is all right at certain times in small amounts, but the time for motion must be carefully chosen, and during the time of exercise the joint must be under constant supervision and the pain and spasm reactions noted.

Another thing which I think it is hard for us to realize is that the use of supports must be continued over considerable length of time even after it seems unnecessary. The after care of chronic arthritis is almost more important, if it is conceivable, than the treatment during the active stage. I am sure that if the acute joints are treated by complete rest, pain, swelling, and permanent damage are decreased. I think that those who have tried this protective rest method of splinting will agree that far less analgesics are necessary where joints are protected and the mental hazard of pain is eliminated to a large extent.

The second benefit which we may expect from orthopedic treatment is that many of the early contraction deformities of the joints can be straightened out without surgical interference, if corrective splints are used. The same method as is used in the prevention of deformity can be carried out over a period of time, if a series of corrective supports are used.

The chief cause of flexion is pain. Pain causes spasm. Spasm causes contraction of the muscles in an attempt to immobilize the joint. If rest is artificially secured, the necessity for spasm is eliminated. The joint relaxes and inflammation subsides. If it has not become too great, the spasm lets go and the deformity is corrected. If deformity has already started it still is not too late to expect some correction by rest and protection, so that orthopedic consultation at this time is still important.

Third, operative procedures to restore functional use of the joints can be instituted with a fair degree of success. Many times there has been a good deal of skepticism as to the possible correction of deformities by operative means. We have been taught that operation was dangerous in arthritis and was rarely successful. I think the reason for this is that we have not operated at the right time, we must wait for the disease to subside, however, and until the patient is able to stand the operation. It has been my experience that operations on arthritic patients were perfectly possible and no more dangerous than in other patients. There seems to be no more likelihood of ankylosis or infection or of lack of bone union in chronic atrophic arthritis than in any other disease, provided the operation is performed after the disease has been quiescent for at least three or four months, longer, if possible, or when the patient has developed a sufficient resistance to the disease and is gaining weight, strength, and vitality. I think it is as wrong to operate on a patient with chronic rheumatoid arthritis orthopedically, as it is to remove a focus of infection at the wrong time and in a state of depleted resistance. One of the operations which has been most successful in correction of deformity in chronic arthritis is synovectomy, for the removal of the synovial tissue which blocks the joint motion. Just as a semihyal cartilage prevents complete extension, so the synovial tissue, being sensitive, produces pain when pinched, and results in flexion. Good motion is often secured by its removal. There are certain dangers in this type of operation, and it is indicated only in a limited number of cases where the anterior part of the knee under the patella in the suprapatellar pouch is filled with masses of inflamed synovial tissue and pannus. There is danger of limited flexion due to adhesions under the patella.

Arthroplasties are often quite successful in elbows and knees but not so good

in hips, shoulders, and fingers. They are indicated where the joints are ankylosed. Good results are usual in elbows and knees. The third operation, the posterior capsuloplasty of the knee, may be very successful where the knee is flexed, only partly subluxated, and where the posterior capsule is very thick, indurated, and too tight to be stretched by manipulation to allow the knee to come out straight. It gives exceedingly stable, straight knees with usually increased range of motion.

The reeducation of muscles should be undertaken prior to operation in order that motion may be secured as soon as possible after the operation. Physical education is vitally important in the after-care of the chronic arthritic, because in walking it is essential that the body be so balanced that there is the least amount of strain on the joints of the legs. This means corrective posture work in order that the trunk may be used normally, that the strain on the hips and knees and feet is the least possible, and particularly that the strain on the neck and back is eliminated. Only through putting the body in the best possible condition can we expect to keep the resistance of the chronic arthritic patient at the high level, prevent the strain on the joints, and prevent recurrence of symptoms.

Flat feet, bent knees, flexed hips—all produce strain and result in back, shoulder, and neck strain. A deformed body is always lacking in resistance. It is true of lateral curvature and is true in arthritis. We have never succeeded in securing satisfactory use of flexed knees. It has always caused trouble sooner or later. A lordotic back always causes pain some time, and strains other joints. Therefore arthritic patients, because of their joint damage, must be particularly protected from strain.

### DISCUSSION

DR. A. R. SHANDS, JR., DUBUQUE, N. C.—The problems which the orthopedist has to meet would be less serious ones if arthritic patients were examined and treated early. The plea which Dr. Swaim is making to the general practitioner is to refer patients for orthopedic care before crippling deformities occur. I am convinced that 75 per cent of the orthopedic work in the correction of deformities in arthritis would be unnecessary if preventive medicine were practiced. In the treatment of these cases the physician must have a tremendous amount of patience and optimism, but the arthritic patient is the most grateful individual for the relief of pain and for the least improvement in joint function.

At the Duke Hospital we have felt that in some patients with contracted knees and elbows a manipulation or stretching of the joints can be accomplished with less force and a greater number of knees can be straightened after fever therapy much more easily than would be expected without this treatment. We have felt that the excessive heat had a softening effect upon the adhesions. Our experience in the treatment of atrophic arthritis has been that very seldom is there permanent relief of symptoms following fever therapy alone.

Dr. Swaim is to be congratulated upon the most excellent results obtained in the treatment of these patients he has shown.

DR. DENIS S. O'CONNOR, NEW HAVEN, CONN.—Dr. Swaim represents the ideal practitioner to handle arthritis. Essentially an internist, he has the viewpoint of the internist, but with his orthopedic training, he is competent to handle the joints themselves while he is bringing the arthritic disease under control. The viewpoint of the internist and the training of the orthopedist in one practitioner is what is needed for the most effective handling of arthritis.

The problem might be divided into the treatment of the disease and protection of the joints. The disease may be, but is not necessarily, mainly in the joints. The effects of the disease are very definitely in the joints.

Arthritis is a medical problem and becomes an orthopedic problem only if the medical attendant fails. Arthritis is an orthopedic problem from the beginning if prevention of deformity logically comes within the province of the orthopedist. There can be no dispute as to the province of the case if the internist is capable of caring for the joints, and it would seem to be within the scope of this Association to survey the teaching of orthopedies in our medical schools to see if the medical practitioner is being taught the basic facts pertaining to joints.

Trauma in relation to arthritis cannot be overemphasized. In true infectious arthritis trauma is frequently the localizing factor. In hypertrophic arthritis, it is an essential etiologic factor. In toxic arthritis it is a sustaining factor. Trauma must be thought of in its widest sense and may be classified as "physiologic," meaning the trauma of physiologic use, "occupational," meaning the trauma which arises from the repeated use of a joint in occupation, and "accidental," representing that violent form which the word trauma usually connotes.

Theoretically the optimum position of use of a joint of correct posture would result in a minimum trauma to the joint but you all know how infrequently the physiologically correct posture is met with in daily life.

The flat feet, the bent knees, the exaggerated spinal curves, the sagging shoulders are faults of posture producing strains. Muscle training with the help of corrective supports will prevent the fixed deformities which usually call forth surgical measures.



## THE TREATMENT OF ATROPHIC (RHEUMATOID) ARTHRITIS WITH LEUCOCYTE CONCENTRATE\*

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THE administration of whole human leucocytes by subcutaneous injection in atrophic (rheumatoid) arthritis patients was suggested to us on theoretical grounds. These theoretical considerations, the method of preparation of the leucocyte concentrate and the results of this type of treatment are presented below.

The research of the last thirty-five years in atrophic (rheumatoid) arthritis tends to show that two factors are operating. One is infection, and the other a fundamental constitutional derangement which allows the infection to produce its characteristic effects. The details of these two factors are still matters of continued dispute.

In the face of undoubted evidence that infection plays an important rôle in atrophic (rheumatoid) arthritis, we find very little change in the circulating leucocytes. It is agreed that the typical blood picture in chronic arthritis is as follows: a slight secondary anemia, a normal leucocyte count, a normal neutrophilic ratio but an increase in the proportion of young, nonfilamented neutrophils, causing a nuclear shift to the left, and in many cases a tendency to lymphocytosis.<sup>1-4</sup>

If infection plays a large rôle in atrophic (rheumatoid) arthritis, it is obvious that the leucopoietic functions of the body do not respond in the usual way. The hemopoietic function is likewise depressed. Anyone with experience in the treatment of arthritis has noted how resistant are the low red blood cell count and hemoglobin to the best possible constitutional treatment. This lack of response on the part of the bone marrow may be due to the effect of some toxin, or, in the case of the leucocytes, to the fact that whatever infection is present in atrophic (rheumatoid) arthritis is so low in virulence that it does not stimulate the bone marrow. Another possibility is that there is a fundamental hormonal deficiency in atrophic (rheumatoid) arthritis of a type simulating the deficiency in pernicious anemia. As far as I know no study of the bone marrow in arthritis has ever been published.

In recent years a great deal of the therapy in arthritis and other apparently infectious diseases of low virulence, has taken the form of injections of heterogeneous substances to produce what is called the nonspecific protein reaction. The most marked characteristic of the reaction is the leucocytosis and neutrophilia. Even the administration of the so-called specific vaccines has this concomitant effect, and it is suspected by many that their sole value

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is in this reaction. While the nonspecific protein reaction is frequently beneficial, it rarely proves curative and so other methods to stimulate the myelopoietic function have been sought.

One of these is the administration of nuclein. Nuclein has been used to stimulate bodily resistance since 1893<sup>4</sup> at first empirically. A rational basis for this use was laid by Jackson in 1924 who demonstrated the existence of pentose nucleotides in normal human blood. Subsequently it was shown by Doan and his coworkers<sup>7</sup> that nucleic acid and its derivatives stimulate the myelopoietic function of bone marrow in rabbits. Doan attributed to these substances qualities of chemotactic, maturative and initiatory stimuli for neutrophilic myelocytes, when the basic mesenchymal tissues from which they arise are in a condition to respond.<sup>8</sup>

Doan and others in 1928<sup>9</sup> and again in 1932<sup>8</sup> summarized the situation to date. The action of these compounds in stimulating the bone marrow probably simulates a normal physiologic mechanism. Sabin<sup>9, 10</sup> showed the presence of nonmotile polymorphonuclear neutrophils in normal living blood. This suggested to Doan<sup>8</sup> the hypothesis that the disintegration products of these cells, one of which is nucleic acid, supplied the stimulus to the bone marrow for the production of new cells. As is well known, clinical application of this theory has been found in agranulocytic angina, where favorable clinical and hematologic results ensue when pentose nucleotide K96 is given intravenously.<sup>11</sup> Reznikoff<sup>12</sup> published the first report on this subject in 1928.

The injection of leucocytes, one of the most important constituents of which is nucleic acid, to stimulate bodily resistance began with Hiss in 1908.<sup>13</sup> He used rabbit leucocytes obtained by pleural injection of aleuronot. After twenty-four hours 30 to 60 cc. of turbid fluid was obtained. This was centrifuged, the cells washed in saline and emulsified in distilled water. Both supernatant fluid and cell residue were used separately and together. Hiss concluded that these extracts had a distinct modifying and curative action when given subcutaneously and intraperitoneally to rabbits and guinea pigs. His results in human epidemic meningitis<sup>14</sup> and lobar pneumonia<sup>15</sup> led him to conclude that he was dealing with "an agent which further clinical test would not unlikely prove of definite therapeutic value."

Subsequently very little was done with leucocytic concentrate until Strumia in 1934<sup>16</sup> claimed to demonstrate that intramuscular injections of this material from human donors into patients with severe neutropenia were followed in most cases by an increase of mature granulocytic cells in the circulation together with clinical improvement. In three normal people treated and studied in this way, none showed an increase of granulocytes or young forms above the normal physiologic fluctuations. Following Strumia's method Davidson and Shapiro<sup>1</sup> used human leucocytic concentrate in one case of neutropenia which followed the use of dimithiphenol.

Another method of leucocyte administration is by whole blood transfusion. It is agreed by all that transfusion stimulates the blood-forming functions. This effect may in large part be due to the disintegration of the injected leucocytes. Transfusions are being used by us in atrophic (rheumatoid) arthritis with definite beneficial but certainly not curative effect.

We decided to use leucocyte concentrate in atrophic (rheumatoid) arthritis because of the nucleic acid content of these cells, and because it was thought that normal leucocytes might contain a hormone lacking in patients with atrophic (rheumatoid) arthritis.

#### PREPARATION OF LEUCOCYTE CONCENTRATE

The leucocyte concentrate was prepared in the following manner. A healthy donor was selected and the presence of venereal and other active infection was eliminated. Three hundred cubic centimeters of blood were aspirated by venipuncture into sterile bottles containing 50 c.c. of physiologic saline and 20 c.c. of 10 per cent sodium citrate. This material was centrifuged for one hour at 2,600 revolutions per minute in tubes one inch wide by six inches long. After removing the serum, the supernatant leucocyte layer was drawn off in sterile Pasteur bulbs. This material was pooled, again centrifuged in the same manner, and the supernatant leucocyte layer was drawn off and placed in rubber-capped vaccine vials. From 300 c.c. of whole blood the yield of leucocytes, partially mixed with red blood cells, was from 25 to 30 c.c. Forty-eight-hour sterility tests were made.

This material was kept on ice and 3 to 6 c.c. was administered to each patient intragluteally, in some cases three days a week, in some seven days a week. The treatment was kept up from three weeks in one case to nine months in the case longest under observation. The local reaction about the site of the injection was negligible. There were no evidences of general reaction except in one instance, where it was subsequently found that the leucocyte concentrate was contaminated.

#### METHOD OF ADMINISTRATION

Ten patients with atrophic (rheumatoid) arthritis were selected by the usual criteria. Their condition ranged in severity from marked generalized, crippling deformity in a child ten years of age, to mild but definite arthritis in elderly subjects. A summary of one case will give an idea of the procedure used.

CASE 1.—(No. A95348.) Female, aged twenty-eight. Diagnosis: atrophic (rheumatoid) arthritis. Symmetrical, generalized periarticular swelling with marked limitation of motion and pain on motion of hands, wrists, elbows, shoulders, and knees. Agglutination reaction for *Streptococcus hemolyticus* AB<sub>12</sub> and NY<sub>6</sub> positive to dilution 1:320. Initial sedimentation rate 49 mm. per hour. The blood count was as follows: red blood cells 3,460,000, hemoglobin 68 per cent, white blood cells 9,300, polymorphonuclear neutrophils 64 per cent, mononuclears 4 per cent, lymphocytes 32 per cent.

On Sept. 13, 1934, 1 c.c. of leucocyte concentrate was administered intragluteally, and rapidly increased by almost daily injections to 3 c.c. and thereafter varied from 3 to 6 c.c.

In four weeks there was marked improvement in the patient's condition. She was decidedly stronger, had less pain, and almost unlimited motion of the upper extremities. She resumed her household duties for the first time in a year. She discontinued the use of aspirin, whereas formerly she had taken up to 45 gr. a day. She noted a decrease of pallor in her skin, especially her hands.

After the first month progress was less marked. Once she had urticaria for one week. Improvement has been irregular but continuous, so that on the last examination on May 17, 1935, she had unlimited motion and was free from pain except in her knees. Up to this date she had received 412 c.c. of leucocyte concentrate.

During treatment blood counts were repeated. There was no change either in the leucocyte or in the erythrocyte count. The sedimentation rate fluctuated from 24 to 49 mm an hour, the last test on April 30, 1935, being 40 mm.

The other nine patients were similarly treated, although the duration of treatment was less. A summary of the therapeutic results in these cases is given below. It is important to point out that although one of the theoretical reasons for the administration of the leucocyte concentrate was to stimulate the bone marrow, in no case was a change in the leucocytes observed above that attributable to the normal physiologic fluctuations. In one case where many counts were made throughout two days the same results were obtained.

#### SUMMARY OF RESULTS

Ten cases of atrophic (rheumatoid) arthritis have been treated with leucocyte concentrate. No case can be considered cured. Six patients show decided symptomatic and general constitutional improvement. In the remaining four patients improvement was only slight or questionable. Improvement consisted of a decrease in pain, an increase in joint motion, and a feeling of increased strength and well being. These changes were not, however, accompanied by improvement in the blood picture or in the sedimentation rate.

#### DISCUSSION

It is clear to us that intraglutal injections of leucocyte concentrate have a definite beneficial effect in some cases of atrophic (rheumatoid) arthritis. The effect is apparently in no way specific. In view of the lack of change in the sedimentation rate, it appears that its effect is constitutional and only indirectly affects the arthritis. Its mode of action is obscure. The amount of nucleic acid present in the injected leucocytes is not sufficient to affect the blood picture. The beneficial effect may be purely a nonspecific protein reaction but the effect was obtained more quickly, was more lasting and in every way far superior to that obtained by any foreign protein so far used by us.

An attempt was made to use animal leucocytes. Fresh sheep and horse blood was obtained and the leucocytes were centrifuged off in the way described above. The local and general reactions were so severe, however, that this method had to be abandoned.

#### CONCLUSIONS

1. The intraglutal injection of whole human leucocytes into patients with atrophic (rheumatoid) arthritis is followed in some cases by prompt improvement in the joint symptoms and in the general well being of the patient.

2. In no instance was the patient considered cured.

3. The injections of leucocyte concentrate did not change the total circulating leucocytes, or their relative proportions beyond the usual physiologic fluctuations. In no case was the sedimentation rate significantly or permanently changed.

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## THE USE OF CINCHOPHEN IN THE TREATMENT OF CHRONIC ARTHRITIS

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**INTRODUCTION**—Arthritis is the most disabling and costly of all the chronic diseases. It was known to the ancients and numerous remedies for its relief have been advocated down through the ages. Its incidence today is of no less importance than that of a generation ago. It is estimated that in this country arthritis is more prevalent than tuberculosis and heart disease combined.

During the past fifteen years rapid advances have been made in our methods of treating this malady. This is to a large extent the result of a better understanding of its classification and of the various etiologic factors that tend toward the persistence of the disease. Since no single etiologic factor has been discovered as the sole cause of any of the various types of arthritis it naturally follows that we must utilize to the fullest extent every efficient method we have at our disposal for combating its onset and progress. While not in any way wishing to detract from the credit which should be given to such well known methods of treatment as rest, dietary measures, removal of focus of infection, physiotherapy, posture, climate, etc., the authors feel that the drug treatment of arthritis during recent times has not received the proper degree of attention which it justly deserves. It would appear that perhaps this may be due to four factors. (1) The dosages employed in most instances have not been large enough to secure the desired therapeutic result because most physicians use minimal doses, whereas the method of choice is to increase the dose gradually up to the point of intolerance. (2) In many instances the use of medication has not been persisted in for a sufficient length of time. (3) The prevailing impression of the medical profession is that the relief seems to be due entirely to the analgesic effect and they have entirely neglected to take into consideration the beneficial effects on the kidney and the liver in stimulating increased elimination of nitrogenous waste products. Finally, (4) Cinchophen, our most important drug, has been too hastily condemned by many physicians because of reports in the literature which seem to indicate that this drug and its derivatives are toxic to the liver and kidney, because many deaths from acute yellow atrophy have been attributed to its use. In spite of this increasing apprehension the authors of this paper have continued to use cinchophen routinely in all cases of chronic arthritis over a period of ten years, about 1,800 cases in private practice and 760 in hospital clinics, and have never seen a single case of jaundice from its use during that time. During the past three or four years the authors have met several prominent physicians who have stated that they had given up the use of cinchophen entirely on account of the alleged toxic effect upon the liver. In one hospital an order was sent forth prohibiting any

physician in the institution from prescribing cinchophen. Many other physicians refuse to administer this drug for fear that they may later become involved, from a medicolegal aspect, in case the patient should develop jaundice from any cause. The authors do not contend that cinchophen is an entirely harmless drug, but they feel that the reports of liver damage have been grossly exaggerated and that a careful analysis of these reports would prove valuable in an effort to clear up the conflicting views on the subject.

#### SHORT HISTORY OF DRUG THERAPY IN ARTHRITIS

Brunton's *Therapeutics and Materia Medica* of fifty years ago set forth the merits of over a hundred remedies for the treatment of rheumatism. Foremost among these were colchicum, sodium salicylate, and potassium iodide. Since benefits were attributed to so many different drugs, it was obvious that there was no one which was really effective in all cases. Colchicum, a natural product, for example, in sufficient doses relieved the pain in gout promptly and satisfactorily but caused marked gastric irritation, weakness, and diarrhea. Salicylates (originally obtained from oil of wintergreen although later synthesized) sometimes produced not only irritating effects on the stomach but had the distinct disadvantage of producing renal damage when doses large enough to be effective were employed.

An attempt was made by various investigators to obtain a satisfactory compound which would adequately relieve pain and at the same time not upset the gastrointestinal tract. Cinchophen obviously met these requirements.

*History of Cinchophen Therapy*—Nicholaier and Dohrn<sup>1</sup> in 1908 called attention to the fact that this product was an excellent analgesic and antipyretic in rheumatic conditions. They believed that it definitely increased the excretion of the end-products of protein metabolism, more especially uric acid and urea.

Davis<sup>2</sup> confirmed the fact that it was an excellent analgesic, etc., and also stated that it diminishes congestion of the joints.

Fine and Chace<sup>3</sup> believed that the beneficial effects these patients had were due to the increased permeability of the kidney.

Folin and Lyman<sup>4</sup> demonstrated that the increased uric acid in the urine following the administration of cinchophen was represented by the amount of this substance that had previously accumulated in the blood stream due to the associated kidney insufficiency.

McLester<sup>5</sup> stated that the use of cinchophen diminished the amount of uric acid in the blood 50 per cent and increased the output of uric acid in the urine 300 per cent.

Nicholaier and Dohrn<sup>1</sup> pointed out that the output of uric acid is greatest in the first thirty-six hours after administration and following this period, and remains at the normal level for the duration of the use of the drug. However, they pointed out, if it were discontinued for a few days and then started again, they were able to reproduce an increase in the output of uric acid.

During recent years considerable attention has been devoted to the effect of cinchophen upon the liver. Brugsch and Horsters<sup>6</sup> found that sodium

cinchophen given in doses of from 0.8 to 3 gm. increased the secretion of bile from 8 to 864 per cent. The total quantity of solids, acids, viscosity and surface tension quotients and pigments of twenty-four-hour collections were all increased.

Grubenbergl and Ullman<sup>7</sup> reported increase in bile in the duodenal contents following the administration of cinchophen to healthy individuals as well as patients suffering from catarrhal jaundice. Brugsch<sup>8</sup> reported favorable results following the use of cinchophen in treating jaundice and subacute atrophy of the liver. Lichtman<sup>9</sup> confirmed this statement and showed that single or even repeated doses of 0.45 gm. of cinchophen are not toxic, even in the presence of severe damage to the liver. Mendel<sup>10</sup> observed what Davis<sup>2</sup> later confirmed, that the use of cinchophen diminishes congestion in and around inflamed joints. He believed that this is probably due to the paralyzing effect of cinchophen on the amoeboid movement of the leucocytes. He argued that the increased amount of protein thrown into the blood stream is the result of the destruction of the leucocytes and is sufficient to explain the increased excretion of uric acid following the use of this drug.

*Reports of Toxic Action of Cinchophen.*—In 1913 Phillips<sup>11</sup> first described skin reactions to cinchophen. This report was followed by similar ones from Schroeder,<sup>12</sup> Worster-Drought,<sup>13</sup> Reichle,<sup>14</sup> Cabot,<sup>15</sup> Parsons and Harding,<sup>16</sup> and Rabinowitz.<sup>17</sup> These observers felt that it was a dangerous drug to use; that many people had an idiosyncrasy to it and that even small doses might initiate an attack of jaundice which occasionally proved fatal.

In October, 1932, J. S. Davis, Jr.,<sup>2</sup> compiled an excellent review of all the literature on the subject and also at the same time published the results obtained in a series of 200 consecutive cases in which the patients had been taking cinchophen and neocinchophen. No fatal results were observed. Thirty patients, however, had some toxic symptoms. None of these were of a serious nature, and none of the patients were confined to bed because of symptoms.

In 1931 E. P. C. White<sup>18</sup> made a careful study of twenty-one selected cases of advanced arthritis. All the patients took two capsules of Mono-iodo-cinchophen three times daily for periods ranging from seventeen to twenty-four weeks. She emphasized that, while there have been a number of deaths attributed to the use of cinchophen, there is a relatively small number of proved cases in proportion to the amount of cinchophen and its derivatives consumed in the United States, which was approximately 90,000 pounds a year. She pointed out that the symptoms of toxicity and their severity are not in proportion to the length of time the drug has been taken, nor to the amount of the drug ingested. At no time during the observation period did the extensive laboratory studies made of these twenty-one patients show any variation from the normal. Eaton<sup>19</sup> also confirms these observations. He has treated several thousand cases in the Arthritis Clinics of the Flower and Metropolitan Hospitals of New York, and has never seen a case of jaundice following the administration of cinchophen. He has recently given 6,000 intravenous injections of 7½ gr. of cinchophen combined with 34 per cent hexamethylenetetramine, together with the oral administration of cinchophen in these cases, without any deleterious



rious results. Fine and Chace<sup>3</sup> felt that einchophen and neoeinchophen were the drugs of choice when for any reason it seemed desirable to favor the kidneys.

*Reports on Animal Experimentation with Cinchophen.*—Barbour and Lozinsky<sup>20</sup> of McGill University have demonstrated by animal experimentation the relative toxicity of einchophen and its derivatives. The results of their experiments showed that aspirin in dogs is more than twice as toxic as einchophen, when given by mouth. A careful review of the literature to date fails to reveal any authentic reports of liver damage in experimental animals.

*Reported Cases of Cinchophen Poisoning.*—It would seem well worth while at this point to attempt a critical review of the reported cases of einchophen poisoning and to cite our own clinical experience which covers some 2,560 cases over a period of ten years in an effort to counteract the apprehension now so prevalent in the medical profession with regard to the use of einchophen. Between the years 1913 and 1933 the literature reveals a total of 131 cases of einchophen poisoning, 96 of which can be immediately excluded, 48 of these cases because of fragmentary data and the other 48 cases because there were no autopsies or operative proof to confirm the diagnoses. In the 96 cases of this series which we exclude there were 30 that showed as their toxic symptoms only urticaria. Such cases should not be considered in the same category as those which developed jaundice, acute yellow atrophy, or even those in which death occurred following the alleged use of einchophen. The authors do not deny that in rare instances skin reactions do occur coincident with the use of einchophen, but they have also seen it occur with equal frequency after the use of other drugs, vaccines, and occasionally after colonic irrigations. In our cases, since we use all of these methods of treatment in almost every case, we never could be sure that the urticarial lesion was due solely to the use of einchophen. The urticarial reaction is unpleasant, but it is not dangerous and does not interfere in any way with the successful outcome of the case. It can usually be controlled by the elimination of the drug and the use of adrenalin and/or sodium thiosulphate. Of the remaining 66 cases, it is obvious that the diagnosis, even in the hands of expert clinicians, was so uncertain that no definite conclusions could justly be drawn as to the cause of their jaundice. Consequently, it would be most unfair to consider these cases in any critical review of the reported cases of liver damage alleged to einchophen.

The 35 remaining cases came to postmortem. Ten of these obviously had other causes of death, viz.: 2 followed operative procedures and 8 had a typical cirrhosis of the liver. In the remaining 25, death was attributed to acute yellow atrophy of the liver. A careful study of these cases, however, reveals that in 7 cases other etiologic factors which in themselves could account for the pathologic changes were found in the liver, viz.: (1) abscesses of liver and lungs; (2) previous history of eclampsia; (3) history of recurrent severe attacks of typhoid fever; (4) Wassermann 4-plus; (5) history of having received 25 c.c. antipneumococcal serum followed by a severe serum reaction; (6) inoperable cancer of the cervix; and (7) pituitary tumor. Thus, out of all reported cases of einchophen poisoning in the literature (131) through 1933, in only eighteen (13 per cent) did the pathologic findings support such a clinical diag-

nosis The proof that cinchophen did cause death in these instances hinges upon the single fact that the patients developed acute yellow atrophy coincident with the administration of the drug It seems to the authors that the critics of this drug have assumed a great responsibility in claiming that this is prima facie evidence of guilt on the part of the drug employed because they (the critics) are faced with the undeniable fact that acute yellow atrophy occurred long before cinchophen was ever utilized therapeutically Moreover, acute yellow atrophy occurs with relative frequency and may occur in patients with arthritis who have never had cinchophen It cannot be denied that, when it does occur in arthritis, the onset of acute yellow atrophy may merely be a coincidence

In the state of New York alone in the years from 1923 to 1933 there were 712 deaths from acute yellow atrophy in 7 174 572 hospital admissions The authors take the liberty to assume, after comparing the above figures, that if all cinchophen administration were stopped immediately, acute yellow atrophy would still go on in the general population at the same rate

*Chance Toxic Dose*—In the eight year period from 1924 to 1932 inclusive, there were about 660,000 pounds of cinchophen produced and consumed, representing approximately 660 million doses of 7½ gr each Despite this, during this period there were only 38 reported deaths in the United States attributed to the use of this drug, making the chance toxic dose 1 70 000 000, which is as low as one could reasonably expect for any active therapeutic agent Cinchophen is not a harmless drug, but it is a very effective one and when used with proper care and reasonable precautions, its benefits far outweigh its limitations In reply to a recent query the *Journal of the American Medical Association* stated that, "When he gives it with proper precautions, the physician carries no more liability in the prescribing of this than he does of any other potent agent" Salicylates and cinchophen can safely be administered in very large doses and represent a fortunate combination of both antipyretic and analgesic qualities which make them more suitable, convenient and desirable than the employment of two or more drugs possessing the same actions individually

*Report of Personal Experience with the use of Cinchophen*—The authors have been using cinchophen almost routinely in private practice and in the Arthritic Clinics of the Hospital for Ruptured and Crippled of New York, and the Roosevelt Hospital of New York during the past ten years Not only have no deaths occurred but they have seen only one mild case of catarrhal jaundice and have never seen a case of severe jaundice or of acute yellow atrophy following the use of cinchophen It must be admitted that they have only seen an occasional attack of urticaria These are seldom serious as they last only a few days and are easily controlled by stopping the drug In obstinate cases the attack can usually be quickly terminated by the use of hypodermic injections of adrenalin with or without the intravenous use of sodium thiosulphate In many of these cases the urticaria definitely was proved to be due to some other etiologic factor The authors have used cinchophen combined with urotropin intravenously in a series of fifty cases and have not noted any deleterious or toxic effects

*Our Method of Administration*—The authors usually start the use of cincho-

phen with doses of  $7\frac{1}{2}$  gr. three times daily, but it is important to emphasize that in the beginning of the treatment they insist upon seeing the patients three times a week so that any case of gastric irritation, nausea, or itching may be noted as soon as it develops and all medication discontinued. They have frequently found that at a later date they can start with a smaller dose and continue to administer the drug safely. The patient is not allowed to know what drug he is taking. This control is rigidly enforced as the authors do not believe that cinchophen is a safe drug to be sold over the counter, and feel that probably most of the reported toxic effects of cinchophen have been due to self-medication.

If the use of three tablets of  $7\frac{1}{2}$  gr. each a day is not sufficient, the dose is increased to four a day; but the authors find that this is about the largest dose that can safely be taken by patients over a long period of time. In case the pain is not relieved the medication is reinforced by adding 5 gr. of aspirin three times a day. An additional factor of safety is the fact that a great many of the patients have had colonic irrigations during the time they have been taking the cinchophen. Control of the gastrointestinal tract of the patient tends to facilitate the use of cinchophen in patients who do exhibit signs of gastric irritation from the drug by proper dietary measures and the use of colonic irrigations.

Neocinchophen is generally believed to be less dangerous to use than cinchophen, but larger doses are required to obtain the same degree of clinical improvement. The increased doses of neocinchophen produce the same gastrointestinal and urticarial disturbances which occasionally result from the use of cinchophen. The patients object to ingesting the larger doses of neocinchophen and to the greater cost of this drug. In practice, therefore, cinchophen is the more desirable drug to use.

#### SUMMARY

The history of cinchophen is reviewed. The action of the drug, its usefulness in the treatment of arthritis and in liver diseases is discussed. The toxic actions of cinchophen which are reported in the literature are reviewed. Of 131 cases of cinchophen poisoning reported in the literature between the years 1913 and 1933 only 18 had pathologic findings to support a clinical diagnosis of liver damage following the use of cinchophen. In a critical review of the literature covering these twenty years the authors found that there were only 18 deaths, and that approximately 90 million doses of cinchophen were consumed during this time. They therefore feel that the chance toxic dose is too small to preclude the use of this medication. In 2,500 cases treated by the authors with the oral administration of cinchophen and 50 cases treated by intravenous injection of cinchophen there has been no evidence of liver damage. The authors believe this is due largely to the rigid control they exercise over the use of the drug and to the fact that they see their patients every third day for the first two weeks of its administration. It has been emphasized that they never give over 30 gr. a day. At the same time the importance of keeping the gastrointestinal tract in good condition has been stressed. A review of the literature shows that one group of clinicians

believe that the use of cinchophen is dangerous, and give as their reason its toxic action on the liver, while an equally well qualified group of clinicians maintain that they use cinchophen, both here and abroad, for its stimulating effect on the liver in cases of catarrh or even severe jaundice. The experience of the authors in 2,500 cases would seem to favor the latter view, and would seem to indicate that the fatal cases of acute yellow atrophy were either coincidences, or in some way were due to lack of control in the administration of the drug. The authors therefore believe that the use of cinchophen is justified and carries with it no more danger or responsibility than the use of any other potent pharmaceutical preparation.

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#### DISCUSSION

DR HOMER F SWIFT New York City.—The report of Dr Snyder represents a type of investigation that is much needed in the application of drugs in the treatment of patients with disease. Natural idiosyncrasy or acquired hypersensitiveness not infrequently makes it impossible to give many drugs in therapeutic doses. Indeed some individuals are so sensitive to some drugs that a minimal dose will elicit alarming even fatal, phenomena. The ratio of toxic to therapeutic dose is quite variable in different subjects, and this makes careful study of each case most important. These more or less self evident facts are sometimes forgotten in the enthusiasm of the moment to record the alarming by effects of drugs. While it is important to be familiar with these unfavorable effects it is equally desirable to know how frequently they occur. The data presented by the authors seem to indicate that the serious sequelae from cinchophen are relatively uncommon. But that they do occur we can be quite certain, both from a perusal of the literature and from personal experience. Fortunately we have several chemically unrelated drugs which seem to have comparable action on the symptoms of rheumatism, so that when a patient is encountered who reacts unfavorably to one, we are in a position to switch to another. The relative cost of the different remedies is an item worthy of consideration when prolonged medication is anticipated. Our final decision will be influenced by the consideration of all of these factors.

*The remainder of the paper will appear in the March issue of the JOURNAL*

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## EDITORIAL

### Organization in the Study and Control of Rheumatic Diseases

THE rheumatic diseases remain a great medical, economic and sociologic problem. This fact is emphasized statistically by the recent survey in Massachusetts which revealed that over 3 per cent of the total population were suffering from rheumatic disease, and that the total number of such patients exceeded those afflicted with cancer, tuberculosis and heart disease combined. Very active national and local societies have long waged warfare against the last three diseases with excellent results. Such progress has been the direct outcome of the organization of physicians and laymen to stimulate study, carry knowledge gained to both doctor and patient, and improve facilities for the care of those afflicted with tuberculosis, with cancer, or with heart disease. Tuberculosis has an advantage in being contagious, so lawmakers and the general public usually have been willing to provide hospitals for the care of those having tuberculosis and so remove them from the general population as possible infecting mediums. Cancer by reason of its frequency and of the invariably fatal outcome without successful treatment

always has a great popular appeal especially with respect to any new method of therapy. Widespread educational activities have likewise awakened the general public to the importance of heart disease as a social and medical problem. Rheumatism is at a disadvantage in not being contagious and not being fatal.

Until recently, relatively little had been done in this country in an organized way to stimulate interest in chronic joint disease although European countries have collected excellent statistics concerning the economic importance of the disease usually through state insurance channels. Some countries, as Sweden, have provided excellent hospitals for the study and treatment of chronic arthritis. In 1925, the International League Against Rheumatism was formed to correlate the work of committees which were already active in most of the European countries. International congresses on rheumatic diseases have been held from time to time, the fourth being held in Moscow in May 1934. Another activity of the League has been the publication of an official journal, the *Acta Rheumatologica*, under the editorship of J. van Bijsterveld of Amsterdam.

In 1925, the formation of the American Committee for the Control of Rheumatism was initiated by Dr. Louis L. Wilson of the Mayo Foundation acting at the request of European workers. This committee has been affiliated with the International League but has functioned independently. The present members of the committee are

Walter Bauer  
Ralph H. Boots  
Russell B. Cecil  
A. A. Fletcher  
Russell L. Haden  
Ernest E. Irons  
Joseph L. Miller

G. O. H. Minot  
J. A. O'Reilly  
Robert B. Osgood  
Homer F. Swift  
Hans Zingser  
I. S. Hench, Secretary  
Ralph Pemberton, Chairman

The committee has held numerous meetings to discuss rheumatic problems. One result of these meetings has been to bring some order out of previously existing chaos in reference to classification of the rheumatoid clinical and pathologic types of the disease.

Since it was felt from the beginning that the patient suffers greatly from that lack of application of already proved facts concerning the study and treatment of chronic arthritis, the committee prepared an educational exhibit on arthritis at the Detroit meeting of the American Medical Association in June, 1930. A second exhibit was held in Philadelphia in June, 1931, a third in New Orleans in 1932, a fourth in Milwaukee in 1933, and a fifth in Cleveland in 1934. A brochure prepared by the committee was published by the American Medical Association for distribution to visitors to the exhibit in 1932<sup>1</sup> and 1933,<sup>2</sup> and in 1934<sup>3</sup> a new edition of a primer provided originally at the expense of the committee was printed by the American Medical Association. An educational exhibit was also staged by the committee at the Century of Progress Exposition in Chicago.

The American Committee also initiated a Yearly Conference on Rheumatic Diseases, the first being held in New Orleans in 1932. In 1933, the American Association for the Study and Control of Rheumatic Diseases was formed with the purpose of enlarging and taking over the work begun by the American Com-

mittee. Two meetings of the new Society have now been held. These have been well attended. The papers presented at the meeting in Atlantic City in June, 1935, are printed together in this number of the JOURNAL and testify to the excellence of the program. The proceedings of the Cleveland meeting in June, 1934, were printed by the Association.<sup>4</sup> During the past year, a most excellent review of the present status of the rheumatism problem was prepared by a group selected by the American Committee and published in the *Annals of Internal Medicine* for April, May, and June, 1935.<sup>5</sup>

Such organized activities have stimulated much interest in the rheumatism problem in this country and have given impetus to further study to the end that the sufferer from arthritis may be treated more intelligently and have a much greater chance for recovery. In time, the laity will certainly be aroused to a more active part in the campaign, and it is hoped the time will come when hospital beds will be provided for the proper treatment of chronic arthritis. This can come about only through organized work such as is already under way.

The British Medical Association in 1931 appointed a special committee for the promotion of research into the causes of arthritis and allied conditions and the investigation of methods of treatment. This committee, under the chairmanship of Sir Humphrey Rolleston, has prepared a report<sup>6</sup> on nomenclature and classification and other important features of chronic arthritis along the same lines followed by the American Committee. In 1933, a permanent English Committee on Rheumatic Diseases was appointed by the Royal College of Physicians with Sir Humphrey Rolleston again as chairman, which has been most active. The first annual report of this committee has just appeared.<sup>7</sup>

The value of such organized activities in this field will certainly be reflected soon in a more general appreciation of the rheumatism problem by the rank and file of the medical profession and come back to the patient in the way of more intelligent care. International efforts emphasize the importance of the problem. The activities in the English-speaking countries are especially noteworthy.

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R. L. H.

#### Erratum

In the January issue, article by Greenberg and Miroslubova, Table I, page 134, first line under heading should read:

Human F  $11.5 \pm 0.2$

$11.8 \pm 0.2$

$10.5 \pm 0.25$

$11.55 \pm 0.2$

# The Journal of Laboratory and Clinical Medicine

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## CLINICAL AND EXPERIMENTAL

### THE NATURE OF RHEUMATIC FEVER\*

HOMER F. SWIFT, M. D., NEW YORK, N. Y.

**I**N BEGINNING this symposium it would be well to define the nature of the disease under discussion. This will be attempted so far as contemporary knowledge permits, for it should be recognized that until the etiology is conclusively demonstrated, and specific diagnostic measures are available, most discussions must, of necessity, consist in reviewing theories that knit together current observations.

#### CLINICAL CHARACTERISTICS

The clinical manifestations of a disease often give a clue concerning its nature, particularly when a given pattern is followed closely enough to permit clear differentiation from not too distantly related conditions. Thus, upon first glance, would seem to apply to rheumatic fever and especially to that type formerly known as acute articular rheumatism, with migratory polyarthritis, pyrexia, toxemia, and cardiac symptoms as prominent features. Many variations from this, so called, typical picture exist, in fact they are sufficiently numerous apparently to comprise the majority of cases encountered in a large general clinic where both children and adults are treated. Monosymptomatic manifestations, such as cardiac insufficiency or chorea may predominate, and the accompanying signs of mild infection, with undernutrition, slight fever, low grade leucocytosis, accelerated erythrocyte sedimentation rate and altered electrocardiograms, may require careful documenting before the rheumatic nature of the disturbance can

\*From the Hospital of the Rockefeller Institute for Medical Research.  
Read at the Second Annual Meeting of The American Association for the Study and Control of Rheumatic Diseases and the Fourth Conference on Rheumatic Diseases, June 10, 1935, Atlantic City, N. J.



be determined. In fact, the symptomatology may be so obscure that the pathologist's verdict, derived from microscopic examinations, must be the deciding factor.

Temporal variations are equally outstanding. On the one hand, we may see polyarthritis, fever, and toxicity of short duration; the patient recovers and is apparently free from any further rheumatism for the remainder of his life. On the other hand, one encounters a patient with low grade fever, progressive carditis, occasional congestive failure, continued slight leucocytosis, and increased erythrocyte sedimentation rate lasting for years, until finally acute cardiac failure drops the curtain on a life of chronic invalidism. Again, persistent arthritis may be so marked that the physician is in doubt as to whether he is dealing with a case of acute arthritis deformans or of subacute rheumatic fever. First attacks of chorea may be typical, while subsequent recurrences are not infrequently manifestations of habit spasms. Subcutaneous rheumatic nodules are sometimes the chief signs in a person who considers himself to be in perfect health; and how frequently does the cardiologist encounter full-blown mitral stenosis in patients who deny previous rheumatic manifestations, or does the pathologist find Aschoff bodies in the heart of a subject whose clinical history is likewise free from rheumatic stigmas? A realization of the foregoing possibilities makes the experienced clinician cautious when facing a combination of symptoms that have many of the characteristics of rheumatism and yet may possibly be induced by other infections.

One should also recall the tendency for manifestations to recur, especially during childhood, while after puberty this tendency decreases. Puberty, indeed, seems to constitute an important and critical stage in the life of the rheumatic youth. If, because of previous damage, his heart has undergone marked hypertrophy and is unable to meet the demands of rapid bodily growth, ensuing failure soon terminates the picture. If, on the other hand, the child has sufficient reserve to pass this critical period, he will probably live through succeeding years with distinctly fewer rheumatic relapses than he suffered before the age of puberty. The possible relationship between immunity, or resistance, to rheumatism and the action of sex hormones is worthy of more careful study. In passing, one might mention the apparent connection between certain phases of the menstrual cycle and the appearance of rheumatic relapses seen in some women; it occasionally is observed over periods of many months.

The question of the nature of rheumatic relapses is, as yet, unanswered. Are they the result of introduction of fresh infectious material into a susceptible subject, such as we see in erysipelas; are they due to an altered reactivity of tissues so conditioned that slight insults of various types lead to a peculiar mode of response; or are they manifestations of prolonged residence of some infectious agent in the rheumatic subject with periods of almost complete immunity followed by episodes of diminished resistance, comparable to the relapses one sees in syphilis or malaria? Even in patients who appear to be having a continued infection one encounters phenomena indicating alternate cycles of attempts at recovery followed by periods of lowered resistance.

## NATURE OF THE HISTOPATHOLOGIC CHANGES

The two active inflammatory responses to injury, viz., exudation and proliferation, are prominent features of the rheumatic processes. The former is illustrated in and about an acutely swollen joint, the latter in the subcutaneous nodule, or in the myocardial Aschoff body. While exudative and proliferative are convenient descriptive terms to apply to different pathologic pictures, they do not represent mutually exclusive processes and proliferation often exists in the presence of a rheumatic exudate. Both are responses to injury; the exudate, consisting of plasma, or of synovial fluid and wandering cells, is apparently earlier and more evanescent; the proliferate appears later and is more lasting.

McEwen's<sup>1</sup> studies indicate that the proliferated cells are distinctly different from the epithelioid cells encountered in tuberculosis or from those in syphilitic lesions. They are apparently nonphagocytic and are probably very primitive forms arising from resting mesenchymal cells, although it is possible that at times they may derive from endothelial elements. Their peculiar architectural arrangement in the form of submiliary nodules is claimed by many pathologists to be specific for this disease; other observers regard their presence merely as evidence of a response to injury, and these two schools of thought apparently cannot be reconciled. The Aschoff body when fully developed may present a highly characteristic picture, but often cellular arrangements are such that their true character is not apparent unless compared with the, so called, specific granuloma.

Possibly even more important for an understanding of the pathogenesis of this disease is the altered intercellular mesenchymal ground substance. The collagen fibers show evidence of injury arising from simple focal edema to fibrinoid swelling and focal necrosis. According to Klinge<sup>2</sup> this precedes proliferation of the primitive cells; indeed he thinks that this proliferation is the direct response to alterations in the mesenchymal ground substance. What induces these changes is a matter of conjecture, and were it definitely known we possibly might be much closer to a definite understanding of the etiology of this disease. Klinge thinks that it is a manifestation of tissue hypersensitivity, for he could easily demonstrate fibrinoid swelling in the tissues of rabbits repeatedly injected with foreign protein, and possibly upon this basis one could postulate that the injurious substance was formed by a union of antibody and antigen, presumably of bacterial origin. Fibrinoid swelling, on the other hand, can be induced by bacteria or bacterial toxins, and has also been described in the tissues of noninfected scorbutic animals.<sup>3, 4</sup> Its occurrence, therefore, cannot be definitely attributed to any one type of noxious agent. So far as we know, it has not been induced by filtrable viruses. According to Rivers,<sup>5</sup> all viruses apparently attack primarily either the nucleus or cytoplasm of cells, and the inflammatory reaction is secondary to this cellular injury. We cannot regard the ground substance as cytoplasmic in nature, but rather as an excretory product of the mesenchymal cells; it has more of a supporting than an active metabolic function. If we are correct in assuming that filtrable viruses attack only the active cell bodies or nuclei, it would appear that some other factor was responsible for the focal injury so characteristic of the rheumatic process. C

the other hand, there may be primary points of attack by viruses as yet unrecognized, and if this should be the case, rheumatic fever might be included in this new category.\*

#### STREPTOCOCCI AND RHEUMATIC FEVER

That streptococci may play an important rôle in the causation of rheumatic fever has been the subject of discussion for many years, and recently the possible relationship of hemolytic streptococci has been specially stressed. To review this subject completely would be too time-consuming; hence only the more salient points will be discussed. The long recorded relationship between prodromal tonsillitis or severe nasopharyngitis and rheumatic fever is a matter of common experience. This is especially obvious in adults suffering from their first attack or in those free from the disease for long periods. Following the initial upper respiratory infection there is an apparent quiescent period of a few days to four weeks, the so-called latent period, before the characteristic rheumatic syndrome appears. Unfortunately, careful study of patients during this period is usually impracticable; and in most instances, where carried out, the subjects have been youthful patients in convalescent homes, who have only recently recovered from an attack of rheumatism. Coburn and Pauli<sup>6, 8</sup> have studied nurses, some previously rheumatic and some not. Because tonsillitis is usually associated with heavy hemolytic streptococcal infection, and also because severe nasopharyngitis is often similarly characterized, it has been quite generally assumed that the rheumatic sequellae to these respiratory infections have had these microorganisms as the immediate causative agents. Several observers have shown that precipitins<sup>6</sup> against certain chemical fractions of hemolytic streptococci and also that antistreptolysins<sup>9, 10</sup> and antistreptofibrinolysins<sup>11</sup> appear in the blood about the time of onset of rheumatic symptoms. It has, therefore, been tacitly, if not actively, assumed that some of these antibodies may possibly play a pathogenic rôle in this disease; such an argument is particularly cogent if it is postulated that the pathogenesis rests in part on an allergic basis. Todd<sup>9</sup> originally demonstrated that a large proportion of patients with active rheumatic fever have, what he considered, abnormal concentrations of antistreptolysins in their serums, and therefore concluded that this was highly suggestive evidence of recent hemolytic streptococcal infection. Coburn and Pauli<sup>8</sup> concurred with these conclusions, but reported that the appearance of precipitins against streptococcal proteins was more characteristic of active rheumatic infection than was the occurrence of antistreptolysins.† Most observers agree that high antistreptolysin titers are characteristic of hemolytic streptococcal infections rather than of rheumatic fever. Our own experience,<sup>12</sup> as well as that of others, indicates that precipitins against streptococcal

\*Andrewes has recently described a variant of the rabbit fibroma virus of Shope, which variant induces chiefly inflammatory lesions in rabbits. The evolution of these virus-induced inflammatory lesions has not been minutely described; but Andrewes' observations apparently afford an exception to the general rule laid down by Rivers that invasion and injury of cells by virus precedes inflammation. (Andrewes, C. H.: Viruses in Relation to the Aetiology of Tumours, *Lancet* 2: 63 and 117, 1934; Faulkner, G. H., and Andrewes, C. H.: Propagation of a Strain of Rabbit Fibroma Virus in Tissue-Culture, *Brit. J. Exper. Path.* 16: 271, 1935.)

†Subsequent to the preparation of the present review Coburn and Pauli (Studies on the Immune Response of the Rheumatic Subject and Its Relationship to Activity of the Rheumatic Process. II. Observations on an Epidemic of Influenza followed by H. Infection in a Rheumatic Colony, *J. Exper. Med.* 62: 137, 1935) recorded rheumatic relapses in a group of rheumatic girls in whom the symptoms and ran closely parallel.

proteins are usually demonstrable following streptococcal infections unaccompanied by rheumatism. We are therefore, forced to the conclusion that the presence of all antistreptococcal antibodies thus far described is characteristic of hemolytic streptococcal infection rather than of the rheumatic state, and that none of these antibodies are present either qualitatively or quantitatively, to this rheumatic state.

Wilson and her co-workers<sup>11-14</sup> have, indeed, recently concluded that their data, dealing with the isolation of hemolytic streptococci from the throats of rheumatic and nonrheumatic children and with the occurrence of antistreptolysins in their serums, indicate no relationship at all between streptococcal infections and rheumatic fever. Data so apparently out of accord with the experience of most other investigators merit some comment because neglect of prophylactic precautions based upon a streptococcal theory of etiology (at least contributory) may lead to serious consequences for rheumatic patients. Admitting that hemolytic streptococci were recovered from the throats of most of their patients, one would like to know the kind of streptococci. What were the colonial, pathogenic, or toxigenic characters of the streptococci recovered? To what serologic group<sup>15</sup> did they belong? So far as we now know members of Group A and possibly of Group F comprise the majority of strains probably pathogenic for man. There is reason to believe that human beings often carry members of other groups in their throats with impunity, and, in fact, according to the findings of Lancefield and Hare<sup>1</sup> strains not belonging to Group A can live in such highly vulnerable areas as the birth canals of parturient women without inducing disease. Furthermore these non-Group A strains do not induce antistreptolysins against the soluble hemolysins produced in broth by Group A streptococci.<sup>16</sup> If then for sake of illustration, a patient were carrying Group B strains in his throat this would have no influence on the antistreptolysin content of his serum when tested by the usual methods. As carried out by these investigators the identification of hemolytic streptococci by simple inspection of growths of mixed cultures on blood agar plates is exceedingly difficult, and obviously this technique gives no idea of the serologic group to which the streptococci belonged. Granting however, that the cocci recovered were all members of Group A, this is no assurance that they would induce a rise in the antistreptolysin curve in the carrier's serum, for in our experience roughly 20 per cent of patients definitely infected with proved Group A streptococci failed to show a significant rise in the antistreptolysin in their serums. The carrying of pathogenic streptococci deep in the tonsillar crypts or in the lymphatic glands might escape detection in cultures made from pharyngeal swabbings, but such deeply situated streptococci might easily induce the formation of antistreptolysins in high titers and still the causal relationship be unobservable. Finally, the failure of Wilson's group of children to show the usual seasonal late winter and early spring rise in rheumatic relapses is different from the experience of most observers in New York City. This unusual happening suggests that possibly some prophylactic measures or other factors may have interfered with the customary course of events. It explains, in part, how difficult it is to compare these observations with those of others.

Coburn and Pauli's data and conclusions<sup>7, 8</sup> are the direct antithesis of those just discussed. These observers carefully investigated the streptococci isolated, and identified at least six different types belonging to Group A. Dr. Laneefield has proved the existence of at least ten different serologic types belonging to Group A among the strains isolated from our rheumatic patients; and in Griffith's<sup>19</sup> recent paper fifteen different types from rheumatic individuals were recorded as identified by the slide agglutination technic. As in all of the works just cited the same types were recovered from nonrheumatic patients with streptococcal infections, it seems obvious that none of these strains carry any unique rheumatism-inducing properties.

Coburn and Pauli's numerical data are worthy of citation. During a three-year period, in one-third of their rheumatic subjects there were neither hemolytic streptococcal infections nor rheumatic recrudescences, while in another third the order of events was characterized by an appearance of streptococci in the throat prior to a rheumatic attack. Among the remaining third, one-sixth had relapses unpreceded by hemolytic streptococcal infections, one-fourth had many hemolytic streptococci in the throat without a return of rheumatism, and in the remainder no definite relationship between the two phenomena could be demonstrated. These data, which indicate that although the relationship between streptococcal infection and rheumatism is highly suggestive of having a causal character, it is not by any means absolute.<sup>7, 8</sup>

Schlesinger, Signy and Payne<sup>20</sup> could demonstrate precipitins against certain chemical fractions of hemolytic streptococci in only one-half of their rheumatic children. These authors state that when these antibodies were present they appeared to be related to a complicating streptococcal infection rather than to the rheumatic syndrome, for many patients with typical rheumatic symptoms, but without a recent history of streptococcal infection, failed to develop precipitins. Our own studies<sup>12</sup> of antibodies in the serums of patients taken repeatedly during the course of an attack have failed to show any constant relationship between severity of symptoms and the presence of precipitins against either the "C" (carbohydrate) or "P" (nucleoprotein) fractions of Group A hemolytic streptococci. In this connection, however, it should be mentioned that in animals, at least, there is not necessarily a direct connection between a hyperergic state of their tissues and the presence of antibacterial antibodies in their serums.<sup>21</sup>

\*These authors have very recently stated that although there is strong evidence indicating the importance of infection with hemolytic streptococcus in initiating rheumatic activity, it is their opinion that this is not the only factor underlying the development of the rheumatic state (Studies on the Immune Response of the Rheumatic Subject and Its Relationship to the Activity of the Rheumatic Process III. Observations on the Reactions of a Rheumatic Group to an Epidemic Infection with Hemolytic Streptococcus of a Single Type, *J. Exper. Med.* 62: 159, 1935.)

†Note added at time of proof reading. Very recently these observers noted that patients having streptococcal infections followed by rheumatic recrudescences yielded strains of Group A hemolytic streptococci that were strong erythrogenic toxin and streptolysin producers while from another group of patients infected with streptococci but without rheumatic sequelae the strains of cocci isolated had neither the strong toxin nor hemolysin producing power. They feel therefore, that the capacity of a given strain to produce certain poisons, together with the tendency of the patient to react in a peculiar manner to the infecting strain, are the requisite factors in the final rheumatic reaction. Their most recently expressed hypothesis appears to border closely on the allergic viewpoint discussed below.

Coburn, A. F., and Pauli, R. H. Studies on the Immune Response and Its Relationship to Activity of the Rheumatic Process IV. Hemolytic Streptococcus, Effective and Non-Effective in Initiating Rheumatism. *Clin. Investigation* 14: 755, 1935. Coburn, A. F., and Pauli, R. H. Same series. VI. Significance of the Rise of Antistreptolysin Level in the Development of Rheumatic Activity. *J. Clin. Investigation* 14: 769. Coburn, A. F., and Pauli, R. H. Same series. VII. Splenectomy in Relation to the Development of Rheumatic Activity, *J. Clin. Investigation* 14: 783, 1935.

## RHEUMATIC FEVER AND AN ALLERGIC STATE

Much attention has been paid in recent years to the relationship between allergy (better hyperergy) and rheumatic fever. Because the tests have been largely made with the products of streptococci thought to have some causative relationship to the disease we shall confine our discussion largely to this phase of the subject. Rheumatic patients have a marked degree of hypersensitiveness to streptococcal proteins or vaccines when introduced either intraarticularly or intravenously.<sup>22, 23, 24, 25</sup> Several sets of statistics indicate that this is higher than in any other group of diseases. With one notable exception patients with recent streptococcal infections show a similar heightened sensitivity, this exception is subacute streptococcal endocarditis where the reactivity might be designated as immune hyporexia for indeed these patients show a high degree of resistance to introduction of living streptococci into their tissues. Rabbits subjected to repeated local infections with streptococci of low virulence develop a state of hyperergy somewhat comparable to that seen in many rheumatic patients.<sup>26</sup> Animals immunized intravenously, on the other hand, show a state of hyporexia, also called immune allergy similar to that of patients with subacute bacterial endocarditis.<sup>27</sup> On the basis of these observations we postulated<sup>28</sup> that the differences in the tissue reactivity of patients with rheumatic fever and of those with subacute bacterial endocarditis could be explained by differences in their "ergic" states. Subsequent work has shown that while the immune state in rabbits is rather locally related to the microorganisms with which the animal has been treated the induced hyperergic state is very broad both in respect of the manner in which it can be induced and in the substances with which it may be detected. In other words an animal made hypersensitive to a given strain of streptococci is also hypersensitive to distantly related strains and even to irritating substances nonprotein in nature, and to physical traumatic insults. Of even greater interest is the observation that an animal may be immune to one type of streptococci and still show hyperergic manifestations when tested with some distantly related types.<sup>29</sup> Numerous examples of this so called allergic irritability<sup>30</sup> might be cited for we are learning that some allergic states are not necessarily so specific in all of their manifestations as once was thought to be the case.<sup>31</sup>

These observations may have a direct bearing on rheumatic fever, for they may explain why such a high proportion of rheumatic patients have marked sensitivity to streptococcal products even though other signs of streptococcal infection are absent. Part of this increased sensitivity may be a manifestation of allergic irritability. This is suggested by the findings of Duckett Jones and Note<sup>32</sup> that rheumatic patients show a high degree of sensitivity to intracutaneous injections of rabbit serum. Our experience in confirming those observations has led us to the conception that rheumatic patients are unusually sensitive to many different injurious agents. Wilson's observation<sup>33</sup> that her rheumatic patients were more subject to colds than a control group of a similar social status might be interpreted in a similar manner. An altered state of reactivity of the tissues of these patients possibly rendered them more easily vulnerable to slight infections. If this conception is eventually confirmed it

gives a point of attack in trying to learn how the hypersensitive condition may be successfully removed. We still feel that all of the possible relationships between hyperergy and rheumatism have not been fully explored.

#### POSSIBLE FILTRABLE VIRUS ETIOLOGY

The failure of the previously discussed factors to explain completely and satisfactorily the pathogenesis of rheumatic fever has led many observers to the opinion that the disease is due to an undiscovered filtrable virus. Gräff,<sup>36</sup> in Germany, has actively advanced this point of view for years, and Aschoff and Fahr and their followers have shown a tendency to agree with him, in opposition to Klinge's allergic theory.

We have not been unmindful of the validity of this hypothesis, and during the past fifteen years have tested it in numerous ways. Large numbers of the various species of laboratory animals have been injected with the following materials derived from patients with active rheumatic fever: blood, plasma, arthritic, pleural and pericardial exudates, cerebrospinal fluid, emulsions of subcutaneous nodules obtained at biopsy and of rheumatic tissues obtained post-mortem; nasopharyngeal exudates that had been obtained by washing the areas with Ringer's solution and also absorbed on cotton plugs that had been introduced into the nares of the patient and then transferred directly to the noses of monkeys. We have, in addition, employed culture media set up in different ways that have proved useful in growing known filtrable viruses. These materials have been injected intravenously, intraperitoneally, intracerebrally, intra-articularly, and subcutaneously; and in some instances we have used animals previously or concomitantly conditioned with streptococcal infections or immunizations. Detailed recital of these experiments would be superfluous in this place. Suffice it to say that aside from discovering, or helping to discover, some new diseases of laboratory animals, the results of these tests have been largely negative. Other workers have been equally unsuccessful in transmitting typical rheumatic fever to animals. The most obvious explanations of these failures are either that a virus was not present in the material used for attempted inoculation, or that the animals employed were not susceptible.

Very recently Schlesinger, Signy and Amies<sup>37</sup> have reported the obtaining from pericardial and pleural fluid of small particles that resemble the elementary bodies found in vaccinia, varicella, psittacosis, and some other virus-induced diseases. These elementary-like bodies were obtained by high-speed centrifugalization of exudates obtained from rheumatic subjects post-mortem. When resuspended in formalised saline they were specifically agglutinated in the serum of ten rheumatic patients out of thirty-six tested. Patients yielding these agglutinating serums were in the active subacute or chronic stage of the disease; but the serums of patients with quiescent rheumatism were nonagglutinating; neither were those of patients with a number of other diseases.\*

This interesting report contains the first positive evidence suggesting that a virus may have an etiologic relationship to rheumatic fever. It is noteworthy

\*Very recently Cohen has reported the observation of "virus bodies" in Giemsa stained films of pericardial and arthritic exudates from patients with rheumatic fever, and also in 25 out of 50 control pericardial fluids. No immunologic studies are recorded. *Lancet* 2: 125, 1935.

that antibodies were demonstrated only in serum from patients with active disease

In this connection attention may be directed to the fact that the demonstration of antibodies, or rather the occurrence of what appears to be an antigen antibody reaction, does not necessarily prove that either the antibody or the substance with which it reacts has an etiologic relationship with the diseased conditions under which they occur. For example Tillett and Francis<sup>38</sup> demonstrated precipitins against the C substance of pneumococci in the serums of our patients during the acute febrile phases of rheumatic fever as well as in that of patients with lobar pneumonia and with generalized staphylococcal infections. The high agglutinating capacity of the serums from patients with typhus fever for *Bacillus proteus* X 19 might also be recalled, but typhus is due to a member of the Rickettsia group of microorganisms.\* Hughes<sup>39</sup> has shown that serums taken from monkeys recently recovered from severe yellow fever contain a precipitin capable of reacting with a precipitinogen which occurs in the blood of monkeys acutely ill with yellow fever. He definitely proved that this precipitinogen is not the virus of yellow fever but that it appears to be associated with a protein of the albumin fraction and is entirely independent of the protective antibody resulting from the infection. A similar precipitin, reacting with the precipitinogen in the blood of monkeys, was found in the serum of human beings recently recovered from severe yellow fever infection. In patients with infectious mononucleosis Paul and Bunnell<sup>40</sup> detected antibodies resembling what they described as heterophile antibodies. Recently Bailey and Raffel<sup>41</sup> have shown that these hemolysins and agglutinins for sheep and ox red blood cells are not true heterophile or Forssman antibodies, but are probably the specific response to an antigen having a factor in common with a thermostable component of these erythrocytes, a certain strain of *B. Welchii* and possibly horse kidney.

In only one of these instances, involving bacteria, Rickettsia, filtrable virus, and unknown infectious agent, respectively, has it been shown that the antigen antibody reaction involves part of a specific etiologic agent. Of extreme interest is the demonstration<sup>39</sup> that the tissues of an animal with yellow fever may become so altered that they act as true antigens and stimulate in that same animal the production of antibodies which have specific diagnostic significance, but no direct etiologic bearing. These examples are cited not in an effort to detract from the importance of the observations of Schlesinger and his co-workers, but to indicate the necessity for caution in accepting the evidence presented. These authors, moreover, state that their results simply tend to confirm the hypothesis that a virus may play an etiologic rôle, and suggest that if it does have this function it is only partly responsible for the disease manifestations, in which streptococci may act as the activating agents.

\*Castaneda definitely established that there is an antigenic fraction of carbohydrate nature common to both the *Bacillus proteus* X 19 and the Rickettsia causing typhus fever. This is extractable from cultures of *Bacillus proteus* X 19 in the so-called X form which gives precipitin reactions with both anti *B. proteus* X 19 serum and with the serum of patients who have had typhus fever as well as with that of horses hyperimmunized with Tillett's. The antigenic relationship between *B. proteus* X 19 and *Typhus rickettsia*. 1. A Study of the Well-Like Reaction. J. Exper. Med. 58: 1-1922. 2. A Study of the Common Antigenic Factor. Ibid. 60: 119-1934. 3. A Study of the Antigenic Composition of Extracts of *Bacillus proteus* X 19. Ibid. 61: 289-1935.



The synergic rôle of infectious agents in inducing diseases should, indeed, be kept in mind. The best established example of such a phenomenon is the combined action of a virus and a bacillus in bringing out the active symptoms of swine influenza.<sup>42</sup> The virus of herpes labialis is carried by many a person for years with comparative impunity; but let him contract some other infection, the blisters promptly appear; sunburn or hyperthermic therapy have similar activating effects on this virus; and certain phases of the menstrual cycle are regularly accompanied by the appearance of labial vesicles in some women. Herpes labialis is the best example of a virus-induced disease in which the infectious agent remains in the subject's body for long periods; although many students of this general class of diseases claim that persistence of viruses in the body is a characteristic phenomenon.

The assumption that a virus is etiologically significant in rheumatic fever, but requires some synergic influence for its activation would explain a number of previously observed features of the disease. While streptococcal infections apparently remain the most frequent precursors of an attack, infections such as measles or other exanthemas have been noted to play a similar rôle. We have seen an attack ushered in by antismallpox vaccination, and also other attacks apparently induced by such injuries as a sprained ankle or a bruised wrist. Bland and Duckett Jones<sup>43</sup> have reported that a febrile reaction following the intravenous injection of typhoid vaccine may have an effect similar to that of tonsillitis in setting off a rheumatic relapse, and have observed similar relapses following accidents and operations. The activating relationship of the menstrual cycle has already been mentioned; the parallism with herpes is obvious. Whether allergic irritability may result from virus infection is, as yet, unknown; and whether allergic states, induced by bacterial infections, are specially suitable for the development of some virus diseases must also be decided by future investigations.

In any event there is much evidence indicating that streptococcal infections must be seriously considered both in investigating the nature of rheumatic fever, and in its prevention and treatment. The devastating effect of some streptococcal epidemics in groups of rheumatic patients has been convincingly demonstrated.<sup>44, 45, 46</sup> So far as we have been able to learn from carefully compiled statistics, severe pharyngitis or tonsillitis of apparent hemolytic streptococcal origin is liable to be followed by rheumatic relapses in 50 to 60 per cent of previously rheumatic subjects, while similar upper respiratory infections in nonrheumatic subjects are followed by rheumatic symptoms in less than 10 per cent of instances. These data indicate the importance of protecting the rheumatic subject from these infections; and doubtless the great difficulty in affording this type of protection to the dispensary and ward class of our population, when compared with persons in better economic circumstances, explains, in part, the relatively greater frequency of rheumatic fever among the former group. That good hygienic environments alone do not insure protection from this disease is illustrated by recent investigations in England, where hemolytic streptococcal epidemics among boys in boarding schools were followed by numerous cases of rheumatic fever, just as they are observed among children of the working classes.<sup>47</sup> While upon first glance the placing of rheumatic children in

a tropical environment, where they do not suffer from severe streptococcal infection, would appear to offer useful prophylactic conditions, it is obvious that such extensive traveling and dislocation of family life that this would entail is out of the question for most persons suffering from this disease. It appears, therefore, most important to devise and investigate other means whereby streptococcal infections may be rendered less devastating in rheumatic subjects.

## SUMMARY

Rheumatic fever presents protean manifestations, which, when few in number, often make it difficult to distinguish the disease from closely related conditions; hence it is impossible to characterize the malady too accurately. The histopathologic picture, while presenting the well-recognized manifestations of inflammation, has certain peculiar features, chief of which are damage to the mesenchymal ground substance by some noxious agent the nature of which has not been definitely established; this damage is followed by hyperplasia and multiplication of primitive cells that often assume a definite architectural form. Inflammatory features are seen early in the rheumatic lesion in contrast to the initial cellular injury followed by the signs of inflammation usually seen in virus-induced diseases. The tissue of rheumatic patients appears to be unusually vulnerable to several injurious agents, and especially to substances contained in or derived from streptococci. Possibly this state of hypervulnerability, or allergic irritability, makes the tissues susceptible to the action of a hypothetical specific virus; on the other hand, the state of allergic irritability may be the result of prolonged action of such a virus, and the immediate attack may be merely set off by a bacterial infection or other traumatic insult. While much investigation remains to establish firmly and to correlate many of the phenomena discussed, the following working hypothesis remains to guide prophylaxis: It appears advisable to protect the rheumatic subject from certain bacterial infections as well as from other injurious and depressing influences if he is to be spared the repeated attacks of a disease that eventually lead to permanent cardiac disability, for repeated or recurring infections usually exert a more deleterious influence than does the initial attack.

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(For discussion see page 571)

# THE NATURAL HISTORY OF CHILDHOOD RHEUMATISM IN MINNESOTA\*

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SINCE 1922 I have been in constant attendance at the Lymanhurst Heart Clinic to which the Minneapolis school physicians refer all children who present signs of cardiac disease. To this clinic, too, the school nurses have been instructed to send immediately on their return to school, all pupils who report their absence as having been due to an attack of rheumatism or chorea as well as all children who come to them complaining of leg pains. In 1926 Dowling School, a special school for orthopedic cripples, was opened to cardiac cripples and during the past nine years I have referred to this school 250 children suffering from heart disease. This group has been examined each week during the school year. Because this work has been carried on with the cooperation of the well-organized medical department of the school system, it has been possible to observe these rheumatic patients at the earliest opportunity and excellent follow-up work has also been possible. This study was undertaken to present the natural history of childhood rheumatism in this locality and to estimate the expected number of recurrences in a group of rheumatic children followed over a sufficient number of years. These control data are necessary in order to determine the efficacy of any type of specific therapy which might be tried in the future.

## SUMMARY OF CASES STUDIED

Between 1922 and 1935, 1,868 patients have been examined at Lymanhurst (Table I). Of this number 160 had congenital heart disease, 27 extrasystolic arrhythmia, 23 neurocirculatory asthenia, 677 were found to have no heart

TABLE I  
SUMMARY OF CASES EXAMINED AT LYMANHURST HEART CLINIC  
1922-1935

Congenital heart disease	160
Extrasystolic arrhythmia	27
Neurocirculatory asthenia	23
No heart disease	677
Nonpathologic murmur	241
Rheumatics	713
1. Potential	405
2. Rheumatic heart disease	308
No diagnosis	27
Total	1,868

\*From the Lymanhurst School Heart Clinic and Convalescent Cardiac Home.

Read at the Second Annual Meeting of The American Association for the Study and Control of Rheumatic Diseases and the Fourth Conference on Rheumatic Diseases, June 10, 1935, Atlantic City, N. J.

disease, 241 presented murmurs which were considered nonpathologic in nature. There were 713 patients who gave a history of rheumatism and of this number 308 had valvular heart disease while 405 were considered as cases of potential cardiac disease. In 27 cases no diagnosis was made.

#### SEASONAL INCIDENCE

As has been pointed out by a number of investigators there is a distinct seasonal variation in childhood rheumatism. In Minneapolis, as shown by

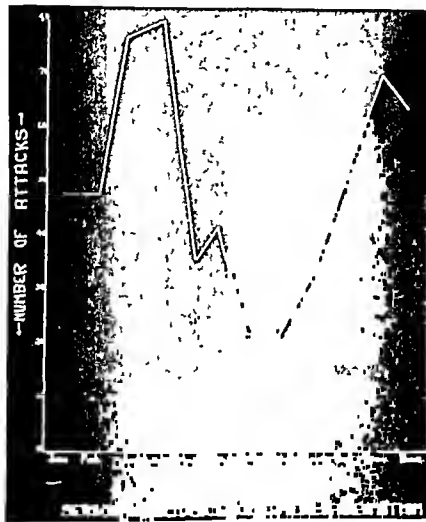


Chart 1

Chart 1, there is a marked increase in the number of cases in the early spring and late fall. During the summer months rheumatic fever is at its lowest ebb. Other cities in the same geographic location have similar but not exactly the same seasonal variations in rheumatic incidence (Chart 2). It will be noted that the seasonal incidence in London, Philadelphia, and New York is practically the same as that in Minneapolis. In Scotland, however, the peak of incidence is in the fall months of October and November with no spring rise. The cause of this seasonal variation is not altogether clear. The seasonal increase in upper respiratory infections, and dietary deficiencies which are more prone to occur at the end of a long winter, have most often been suggested as possible reasons.

## AGE INCIDENCE

Examination of our material indicates as shown by Chart 3 that the onset of childhood rheumatism most commonly occurs between the ages of five and six. This is a somewhat earlier peak of age incidence than is given by most other clinics and is explained by the fact that we have the opportunity of

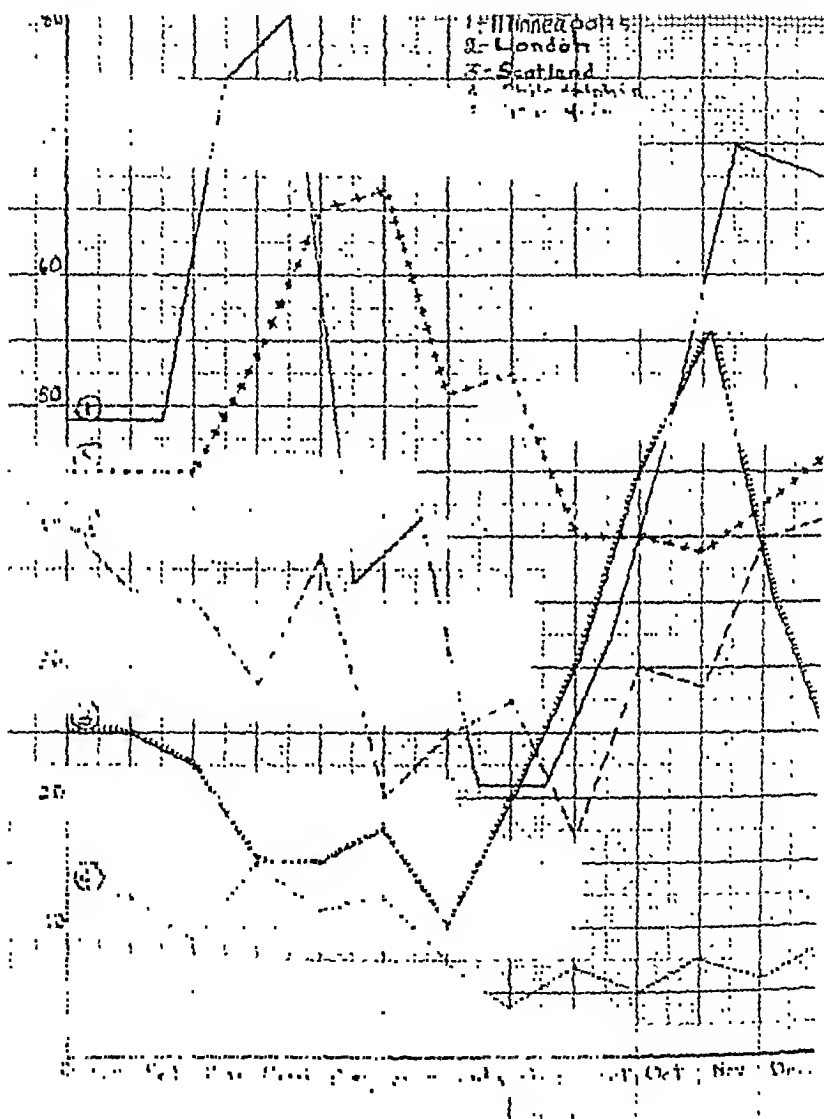


Chart 2.

examining most of our patients at the very onset of the disease. A number of patients have been observed who developed rheumatism during the first few years of life.

## FAMILIAL TENDENCY

It has long been known that rheumatic fever is a markedly familial disease. Because of the importance of this phase of the problem our cases were studied

in this regard. During the year 1933 and 1934 all patients who reported to the clinic were questioned carefully concerning family history. Only the parents and siblings were included and care was taken to include in our data only cases which could be considered as childhood rheumatism. During this period 370 children were examined. Of this number, as shown in Table II, 201 gave a history of rheumatic disease, 120 of these patients already had rheumatic heart disease while 81 were potential cases, 67 had congenital heart disease, 44 nonpathologic murmurs, 5 neurocirculatory asthenia, 6 extrasystolic arrhythmia, and 47 were found to have no heart disease. Of the 201 children

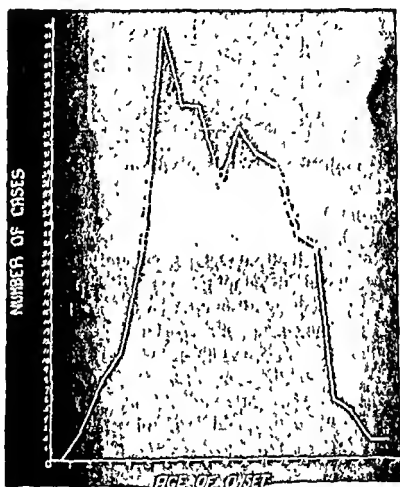


Chart 3

who gave a history of rheumatism, 94 other members of their families gave a positive rheumatic history an incidence of 46.7 per cent, while among the 169 patients who gave a negative rheumatic history, 25 or 14.7 per cent of the other members of their families gave a positive history, indicating that the family tendency among the rheumatic children was three times as great as among the nonrheumatic control group. These figures do not give the complete picture of familial tendency in childhood rheumatism. It is not uncommon to find two or more members of the same family suffering from rheumatic fever at the same time. It has been suggested that rheumatic fever is a mildly contagious disease and a number of so-called epidemics have been described. Discovery of the etiology of this disease will eventually explain the marked familial tendency.



## TYPES OF ONSET

In recent years a good deal has been written concerning the importance of allergy in rheumatism. It has been pointed out by a number of investi-

TABLE II

FAMILY TENDENCY IN JUVENILE RHEUMATISM COMPARED WITH FAMILY TENDENCY IN NON-RHEUMATIC CHILDREN

DIAGNOSIS	CASES	TOTAL NUMBER OF CASES	FAMILY IN- CIDENCE	PER- CENTAGE
Rheumatic heart disease	120	201	94	46.7
Potential heart disease	81			
Congenital heart disease	67	169	25	14.7
Nonpathologic murmur	44			
Neurocirculatory asthenia	5			
Extrasystolic arrhythmia	6			
No heart disease	47			
Total number of cases ex- amined 1933-1934	370			

gators that the great majority of children who develop rheumatic fever have a sore throat or upper respiratory infection a week or two before the rheumatism begins. The usual history, according to these investigators, is that the child develops the upper respiratory infection, apparently recovers, and then a week or two later suddenly develops rheumatism. The quiescent phase is explained as the period of developing sensitivity to the upper respiratory infection. During this past year all our patients have been studied in regard to the type of onset. Time-consuming careful histories were obtained from the patients and their parents. These children for the most part had recently recovered from an attack of rheumatism so the facts regarding onset were still clear in their minds. It was found (Table III) that of 201 children studied 27 or 13.5 per cent had a cold preceding the onset of rheumatism, 9 or 4.5 per cent had a sore throat, 4 or 2 per cent had pneumonia, 2 or 1 per cent had measles, 12 or 5.9 per cent developed rheumatism while convalescing from scarlet fever, 20 or 9.9 per cent had chorea before the rheumatism began, 9 or 4.5 per cent of the patients had various types of onset, 2 of these had infected fingers, 2 had abscessed ears, while 1 child injured himself on falling off a porch about ten days before the onset of an attack of rheumatic

TABLE III

TYPES OF ONSET OF JUVENILE RHEUMATISM

ONSET PRECEDED BY:	NUMBER	PERCENTAGE
Cold	27	13.5
Sore throat	9	4.5
Pneumonia	4	2.0
Measles	2	1.0
Scarlet fever	12	5.9
Chorea	20	9.9
Other	9	4.5
Gradual onset	90	44.8
Not obtained	28	13.9
Total	201	100.0

fever. However, 90 or 44.8 per cent of this group developed rheumatic fever gradually, with no preceding respiratory or throat infection. In many instances the patients and their parents were under the impression that the first involved joint was due to an injury while playing. A knee or ankle became swollen, this condition remained unchanged for a number of days while the child continued in school until gradually other joints became involved and the patient was forced to bed with a well developed attack of rheumatic fever. As a result of this study one concludes that rheumatic fever may be preceded by an upper respiratory infection but the most common type of onset is a gradual one not preceded by any infectious process.

#### RHEUMATIC AND NONRHEUMATIC LEG PAINS

All investigators concerned with the problem of rheumatic infection in children are troubled considerably by the history of leg pains. It is difficult to determine which of these children complaining of leg pains are suffering from rheumatic infection and are in danger of developing rheumatic heart disease, and which have no rheumatic infection. It has long been taught that all children who complain of leg pains are rheumatic and potential cardiacs. The public especially has been led to believe that such leg pains are of serious moment, they have been informed that there is no such thing as "growing pains" but that these pains are rheumatic in nature. As a result, numerous such children have had their tonsils removed, some have been confined to bed for long periods of time because of "beginning heart disease" and no doubt a good number of them have had convalescent home care. As a result of our experience over the past twelve years it is our impression that the great majority of children who complain of leg pains are not suffering from rheumatism and are in no danger of developing heart disease. In an attempt to classify children who complain of pains in the extremities, a differential diagnostic table has been worked out (Table IV) based on clinical observation. A group of known rheumatic children attending Dowling School have been studied in regard to the type of leg pains from which they complained, and these findings have been compared with the symptoms presented by children complaining of leg pains only and who have never had rheumatic fever. Known rheumatic children have more or less constant pain in their joints even while they are up and about and are apparently well. They complain mostly during the day, the pain is intensified on motion, there is difficulty in walking and a limp is often noted. These symptoms ordinarily disappear when the patient goes to bed and rarely do these rheumatic children complain during the night. On the other hand, nonrheumatic children complaining of leg pains have most of their difficulty after going to bed, they commonly awake two or three hours after retiring, and the pain is intense enough to cause them to cry. Heat or massage of the muscles usually causes the pain to disappear, and the child is again able to fall asleep. On arising in the morning the nonrheumatic children are free from pain and complain of no stiffness on walking. The rheumatic children, on the other hand, while they do not complain of leg pains during the night have difficulty in walking when first arising and complain of stiffness for an hour or two in the morning. The

rheumatic children while they may also have muscle pain usually point out the maximum point of pain in the joints themselves, while the nonrheumatic group complain mostly of pain in the muscles of the lower extremities and are unable to locate any point of maximum pain. On being asked to locate his pain the nonrheumatic child will run his hand over the entire lower extremity while the rheumatic patient points directly to his knee, ankle, or elbow joint. The nonrheumatic patient rarely has pain in the upper extremities, the rheumatic child almost always complains of difficulty in all four extremities at one time or another. The nonrheumatic child complaining of leg pains is usually in good health and commonly comes from families in better economic circumstances while the known rheumatic child is usually in poor health and comes from poorer families. The rheumatic child presents other signs of rheumatic activity such as low-grade fever, frequent nosebleeds, pallor, unexplained abdominal cramps, and undernourishment. Frequently, examination of the involved joints in the rheumatic children will reveal local increased temperature, no such findings are present in the nonrheumatic group. A large group of patients complaining of leg pains have been observed for a number of years and will be reported at a later date. It can be noted here, however, that this clinical differentiation has worked well in our experience as almost without exception the children considered as nonrheumatic have not developed signs of cardiac disease. The necessity for differentiating the rheumatic from the nonrheumatic child becomes more and more important as our facilities for treating early cases improves. In a short experience in our Convalescent Home at Lymanhurst we have had to face this problem practically. If care is not taken in selection of patients, a good number of

TABLE IV

DIFFERENCES BETWEEN NONRHEUMATIC "GROWING PAINS" AND JOINT PAINS OF SUBACUTE RHEUMATIC FEVER

	NONRHEUMATIC "GROWING PAINS"	JOINT PAINS OF SUBACUTE RHEUMATIC FEVER
Time of pain	Soon after going to bed. Pain gone in morning. No pain on motion.	Worse on arising. Exaggerated on motion. Difficulty in walking, may cause limp. Pain present during most of day, disappears on getting warm in bed.
Location of pain	In muscles of thighs and legs. Child vague in pointing out site of pain.	In joints themselves. Child points directly to knees or ankles. Often complains of pain in joints of upper extremities also.
General health	Usually good	Usually poor
Other signs of rheumatic activity	Usually none	Common, may have frequent nosebleeds, unexplained fever, pallor, abdominal cramps, undernourishment. Evidence of carditis.
Objective findings in joints	None	Often joints are slightly swollen and hot
Family history of juvenile rheumatism	Uncommon	Very common

nonrheumatic children will be admitted to convalescent homes where they do not belong. The differential diagnosis between the rheumatic and non-rheumatic child is not always easy and it is our impression that this chart based on clinical observations will tend to assist in making this differentiation.

#### EXPECTANCY OF RECURRENCES

In order to determine whether or not any specific type of treatment is effective in such a chronic infectious process as juvenile rheumatism, it is necessary first to know what may be expected in a group of untreated patients followed over a number of years. The normal expectancy of recurrences in a group of children with rheumatism has therefore been studied. As indicated in Table V of a total number of 342 rheumatic children 178 or 52.1 per cent were found to have had one attack only while 164 or 47.9 per cent of these children had recurrent attacks. Of the 164 patients with recurrences it was found that 44 or 26.8 per cent recurred at the end of the first year while a similar number had recurrences after two years, making a total of 88 patients or 53.6 per cent who had recurrences after two years. Twenty-eight or 17.1 per cent of these children recurred at the end of three years, 20 or 12.2 per cent after four years, and 5 or 3.1 per cent after five years making a total of 141 children or 86 per cent who had recurred at the end of five years. As the result of this study, it is fair to conclude that it will be necessary to

TABLE V

#### INCIDENCE OF RECURRENCES IN JUVENILE RHEUMATISM

Total number of rheumatic children	342
Children with one attack only	178 or 52.1%
Children with recurrent attacks	164 or 47.9%

RECURRENCES WITHIN	NUMBER	PER CENT	PER CENT
1 year	44	26.8	53.6
2 years	44	26.8	53.6
3 years	28	17.1	70.7
4 years	20	12.2	82.9
5 years	5	3.1	86.0
6 years	0	0	86.0
7 years	0	0	86.0
8 years	0	0	86.0
9 years or more	0	0	100.0

follow a group of treated rheumatic children a minimum of five years before any definite conclusions can be drawn. In a number of instances reports have occurred in the literature, giving results of treatment with vaccine for two or three years. As indicated by these figures such reports cannot be altogether conclusive until these patients have been followed a minimum of five years. A detailed study has been made of recurrences in our rheumatic children so that we have excellent control material to use for comparison in any group of children treated in the future.

#### ANALYSIS OF PATIENTS DEAD OF RHEUMATIC DISEASE

Table VI gives a résumé of the findings in the 34 children of our group that are known to be dead. Undoubtedly the follow up study which we are

now carrying on will discover many more deaths. It will be noted that in nine instances while these patients were known to have died of rheumatic heart disease, no history of rheumatism was obtained, so that the length of illness from the beginning of the rheumatic process to death could not be

TABLE VI

NO.	SEX	AGE AT DEATH	CAUSE	AGE AT FIRST ATTACK	RECUR-RENCES	TON-SILS	X-RAY	DIAG-NOSES	FAM-ILY	TIME ILL	YEAR DIED
1	M	7-6	R.H.D.			In	+	M.R. + M.S.	-	None	25
2	F	11-6	R.H.D.			In	++	M.R. - M.S. - A.R.	+	None	27
3	M	17-10	R.H.D.	2-1 (Rh)	1. 6-11 (Ch)	6-7	+++	M.R. + M.S.	+	15-9	29
4	M	15-1	R.H.D.	9-2 (Ch)		In	++	M.R. + M.S.	+	5-11	25
5	M	12-10	R.H.D.	4-9	1. 5-7 2. 11-4	In	None	M.R. + M.S.	-	8-1	25
6	F	17-5	R.H.D.	9-5	1. 12-7	9-6	++	M.R. + M.S.	-	8-0	32
7	F	13-2	S.A.B.E.	9-0	1. 13-0	5-3	++	M.R. + M.S.	+	4-2	28
8	M	13-11	R.H.D.	10-0	2. 10-11	2-5	+++	A.R. + A.S. + M.R. + M.S.	-	3-11	29
9	F	14-9	R.H.D.	9-8		3-1	+++	M.R. + M.S.	-	5-1	34
10	M	16-10	R.H.D.	9-6	1. 11-2	6-3	+++	M.R. + M.S.	-	7-4	34
11	F	9-7	R.H.D.	4-9 (Ch)	1. 8-7 (Rh & Ch)	In	+++	M.R. + M.S.	+	4-10	30
12	F	18-0	R.H.D.			8-6	++	M.R. + M.S.	-	None	34
13	M	12-1	R.H.D.	7-10 (Rh & Ch)	1. 11-10 (Rh)	In	-	M.R. + M.S.	-	4-3	27
14	F	15-10	R.H.D.			In	+++	M.R. + M.S.	+	None	26
15	F	13-4	S.A.B.E.	7-0		9-11	-	Potential	-	6-4	32
16	M	15-2	R.H.D.			7-11	++	M.R. + M.S.	-	None	32
17	F	12-9	R.H.D.	6-10	1. 7-7 2. 9-0 3. 11-1	9-5	++	M.R. + M.S. + A.R.	+	5-11	32
18	F	20-1	R.H.D.			Out	+++	M.R. + A.R.	-	None	32
19	M	16-8	R.H.D.	7-7	1. 9-10 2. 10-9 1. 8-7	11-2	+++	M.R. + A.R. + A.S.	-	9-1	30
20	M	8-9	S.A.B.E.	4-7		8-7	-	M.R. + M.S.	-	4-2	34
21	F	17-7	R.H.D.	12-9		15-7	+++	M.R. + M.S.	-	4-10	29
22	M	16-7	S.A.B.E.	13-9		14-0	+++	M.R. + M.S.	-	2-10	29
23	M	15-11	R.H.D.	12-11		10-9	+++	M.R. + M.S.	-	3-0	27
24	M	15-5	R.H.D.	8-5 (Ch)	1. 10-6 (Ch)	8-6	+++	M.R. + M.S.	-	7-0	29
25	M	14-6	R.H.D.	9-6		In	None	M.R. + M.S. + A.R.	-	5-0	27
26	F	14-10	R.H.D.			In	+++	M.R. + M.S.	+	None	30
27	M	11-1	R.H.D.			8-9	-	M.R. + M.S.	-	None	23
28	F	5-4	R.H.D.			In	+	M.R.	-	None	27
29	F	13-8	R.H.D.	10-5	1. 10-9 2. 13-7 1. 7-10 2. 8-11 3. 10-9	In	+	M.R.	-	3-3	33
30	F	12-6	S.A.B.E.	5-11 (Ch)		6-8	++	M.R. + M.S. + A.R.	-	6-7	30
31	M	11-4	R.H.D.	10-5		8-4	None	M.R. + M.S.	-	0-11	31
32	M	14-2	R.H.D.	10-10	1. 11-4 (Ch) 2. 12-3 3. 14-1	11-2	++	M.R. + M.S.	-	3-4	29
33	F	16-2	R.H.D.	9-0		9-4	++	M.R. + M.S.	+	7-2	33
34	F	13-5	R.H.D.	6-9	1. 12-7 (Rh & Ch)	8-8	+	M.R. + M.S.	+	6-8	33

determined. Five patients with rheumatic heart disease died of subacute bacterial endocarditis. Most of these children had repeated attacks of rheumatic fever and chorea, the great majority of them had greatly enlarged hearts as indicated by x-ray examination. The average number of years that these patients lived after the onset of rheumatism was about six years. Two thirds of these children who died had had their tonsils removed.

#### CONCLUSIONS

1 Rheumatic disease in children in Minneapolis is essentially the same as in other centers throughout the world.

2 Rheumatic fever is more prevalent in Minneapolis in the early spring and late fall. During the summer the disease is at its lowest ebb. Similar curves from other large centers are presented.

3 Childhood rheumatism occurs most commonly between five and six years of age.

4 This disease is definitely familial. The familial tendency is three times as great in a rheumatic group as in a nonrheumatic group.

5 Rheumatism in children in a considerable number of instances follows an upper respiratory infection but usually develops slowly with no preceding infectious process.

6 The great majority of children who complain of leg pains are not suffering from rheumatism. A differential diagnostic table based on clinical observations is presented.

7 The normal expectancy of recurrences of rheumatism has been determined. This material is to be used as a control in determining the efficacy of any type of specific treatment which might be tried in the future.

8 An analysis of the findings of 24 children who have died is presented.

#### DISCUSSION ON PAPER OF DR. H. I. SWIFT, "THE NATURE OF RHEUMATIC FEVER," AND PAPER OF M. I. SHAPIRO, "THE NATURAL HISTORY OF CHILDHOOD RHEUMATISM IN MINNESOTA."

DR. JOHN WICKOFF, New York, N. Y.—The very careful study of Dr. Shapiro is one of the many which have built up the clinical picture that Dr. Swift has so ably reviewed. Each such study has added some new knowledge. Of special interest is Dr. Shapiro's study of the various types of pain, especially in the legs of growing children, some of which are probably rheumatic and some of which are not.

About every two or three years we have learned to expect a comprehensive survey of rheumatic fever by Dr. Swift, a master of the subject. He again brings to our attention certain things which we must hold in our minds: the fact that the disease is extremely complex, and that it is very difficult to say clinically what is rheumatic fever and what is not. He has reviewed the liter studies on structural change and has discussed the etiology. I wonder how many times Dr. Swift has come to Atlantic City and has had to listen to a new idea on the etiology of rheumatic fever, has had to set in a receptive mood, and not appear bored.

DR. RALPH KINSELLA, St. Louis, Mo.—I feel personally much indebted to Dr. Shapiro for discussing the subject of growing pains. He has made a comprehensive contribution to it.

It is difficult to discuss the subject of Dr. Swift's paper, the etiology of rheumatic fever. My experience is limited to that which concerns the relationship of streptococcal infections with rheumatic fever.

Not every infectious disease has the pattern of a simple, unique reaction such as lobar pneumonia has. There are many infections which have more than one phase, and a capacity for two phases is easily seen in many coecal infections. The double pattern was forecast in miniature, so to speak, in the experiment of Dr. Swift in which he observed a primary exudative reaction to the intradermal injection of living streptococci and a secondary proliferative reaction in the same site without any further manipulation of the animal. The proliferative reaction may continue in the animal due to the continued presence of the exciting agent.

There is no experimental infection as similar to a rheumatic infection, as far as topographic arrangement is concerned, as one by *Streptococcus hemolyticus* or non-hemolyticus. It is possible to establish a chronic infection in animals, in the case of dogs, by infecting the heart valve and in the case of rabbits, by infecting the knee joint; one can keep a continued infection in the animal's body in this way. In the course of time there will be developed proliferative lesions throughout the body that assume a topographic and histologic arrangement like acute rheumatic fever. Some believe it to be identical with rheumatic fever. However, I think the two conditions remain sharply separated as yet.

The proliferative stage of rheumatic fever or of any disease, is probably one that depends for its continuance on the continued presence in the body of the exciting agent; in this case not yet discovered. This mechanism of reacting in a proliferative way seems to be easily set off in some subjects and follows quickly upon the injury to cells produced by virus. The studies of Dr. Shapiro invite the thought that probably children have some change in their internal environment, as far as bacterial agents are concerned, which may depend on hereditary factors.

## THE RELATIONSHIP BETWEEN RHEUMATIC FEVER AND RHEUMATOID ARTHRITIS\*

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**D**URING the past century there existed considerable controversy over the relationship between rheumatic fever and rheumatoid arthritis. Opinion was more or less equally divided into two schools, those who believed that the two were separate and distinct diseases and those who maintained that they were different expressions of the same fundamental process. At the present time the majority of clinicians both in this country and in Europe consider these two clinical entities as distinct diseases having little or no relation to one another. Occasionally, however, the opinion is still expressed that the two are intimately related and possibly different manifestations of the same process. This is particularly true in the German literature where the relation between the two conditions is constantly discussed.

*Older Conceptions.*—The evolution of opinion regarding the relationship of rheumatic fever and rheumatoid arthritis is of considerable interest. Bal-lonius,<sup>1</sup> in 1642, seems to have been the first to differentiate between rheumatism and arthritis but it was largely due to the teaching and writings of Haygarth<sup>2</sup> (1805) and Heberden<sup>3</sup> (1810) that the two came to be regarded as separate diseases. Even at this early date, however, contrary opinions were held and, in his excellent description of rheumatoid arthritis, Scudamore<sup>4</sup> (1827) stated that this disease was merely a variety of "chronic rheumatism." During the remainder of the nineteenth century opinion as to the relationship of the two seems to have been more or less evenly divided between the two schools. Among those who believed that they were distinct entities may be mentioned Fuller<sup>5</sup> (1832), Adams<sup>6</sup> (1857), A. B. Garrod<sup>7</sup> (1859), Fagge<sup>8</sup> and Bristowe<sup>9</sup>. Contrary opinions were expressed by Watson<sup>10</sup> (1836), Todd<sup>11</sup> (1843), Hutchin-son<sup>12</sup> (1881), Charcot<sup>13</sup> (1881), Sir Dyce Duckworth<sup>14</sup> (1884), Mitchell Bruce<sup>15</sup> (1894), and Hawthorne<sup>16</sup> (1900). Todd, in 1843, stated: "Chronic rheumatism of the joints is clearly traceable to a rheumatic state of the constitution." Duckworth, in 1884, wrote: "The disease (rheumatoid arthritis) is a true form of rheumatism" and "one of the several manifestations of the rheumatic branch of the basic arthritic diathesis." According to Mitchell Bruce, "the two diseases are expressions of one morbid process which differ from each other partly in intensity and partly in the manner of their evolutions." Charcot stated: "There are not two fundamentally distinct diseases to be dealt with as certain authors fancy,

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but only two manifestations of one and the same diathetic state." In a monograph entitled "Rheumatism, Rheumatoid Arthritis and Subcutaneous Nodules," Hawthorne (1900) discussed at length the relationship between the two diseases. He concluded that the presence of similar subcutaneous nodules in both rheumatic fever and rheumatoid arthritis indicated an intimate relationship between the two conditions.

*Modern Conceptions.*—In more recent times there has been a pronounced tendency on the part of American and English clinicians to regard the two as separate and distinct diseases although occasional contrary opinions are still expressed. Thus Clarke<sup>17</sup> (1915) considers the similar geographic distribution of rheumatic fever and rheumatoid arthritis as being indicative of a relationship between the two. Hare<sup>18</sup> (1928) refers to both rheumatic fever and rheumatoid arthritis under the term "rheumatic disease" and states: "I would advance a more catholic outlook upon rheumatic disease, which, in essentials, remains but one of various inflammations of vascular connective tissue." In a report on "The Relation of Orthodox Rheumatic Infection to Multiple Infectious Arthritis" Coates<sup>19</sup> (1930) presents statistics on the incidence of frank rheumatic manifestations in patients with rheumatoid arthritis and in immediate members of their families. While not drawing any definite conclusions he considers that the figures are, to say the least, significant.

In the German and Scandinavian literature a somewhat different opinion prevails. Here the relation of the two diseases is widely discussed and clinicians have found it convenient to divide rheumatoid arthritis into two groups: (1) primary chronic polyarthritis and (2) secondary chronic polyarthritis. The second group comprises those cases which develop as a sequel to rheumatic fever. The recognition of a group of cases intermediate between rheumatic fever and rheumatoid arthritis is very general among German clinicians and a similar point of view is held by the Scandinavians. Freund<sup>20</sup> believes that, in spite of the similarities between the two, they should be separated for clinical purposes. Fischer<sup>21</sup> is of the opinion that the two are intimately related and that such differences as do exist can be attributed to the varying degrees of immunity possessed by the host. He particularly stresses the fact that there are many transitional cases and that it is frequently impossible to separate the two. Kahlmeter<sup>22</sup> states: "In my opinion, therefore, there is a great deal in favor of rheumatic fever and chronic rheumatoid arthritis being due to a pure infection of the same virus and that the different clinical pictures exhibited depend upon differences in the virulence of the infection and upon the different mode of reaction, temporary or constant, of this virus." Klinge and Grzimek,<sup>23</sup> after extensive investigations on the histopathologic changes in the two diseases, conclude that they are one disease process with different phases and with different clinical and anatomic manifestations in each individual phase.

It is apparent from the foregoing résumé that the current tendency in American and English clinical medicine to regard rheumatic fever and rheumatoid arthritis as separate and distinct diseases does not meet with universal favor. In the present communication evidence will be offered in support of the hypothesis that the two diseases are intimately related and possibly

different manifestations of the same fundamental pathologic process. The following phases of the problem will be considered: (1) Familial relationship, (2) geographic distribution, (3) initiating factors, (4) seasonal incidence, (5) age incidence and clinical manifestations in different age periods, (6) pathologic similarities, (7) immunologic findings.

**1 Familial Relationship**—It is well known that orthodox rheumatic fever exhibits a marked familial tendency. The statistics of different authors vary somewhat but careful studies show that more than one member of the family is affected in between 37 and 50 per cent of cases (Diaper and Seegal,<sup>24</sup> Faulkner and White,<sup>25</sup> St Lawrence<sup>26</sup>). It is somewhat less generally appreciated that rheumatoid arthritis also shows a definite familial incidence. In a recent study of 100 cases in our clinic an immediate member of the family was similarly affected in 15 instances. Of more significance, however, is the fact that rheumatic fever and rheumatoid arthritis tend to occur in the same families. In a study of the family history of 50 cases of rheumatoid arthritis, Cortes<sup>19</sup> found that in 16 cases (32 per cent) an immediate member of the family suffered from rheumatic fever. In our own series of rheumatoid arthritis cases the incidence of proved, orthodox, rheumatic infection in the immediate family was 14 per cent. Although considerably less than Coates' figures this is at least fifteen times greater than normal expectancy. It must therefore be concluded that rheumatic fever and rheumatoid arthritis tend to occur in the same families. This observation may be interpreted in a variety of ways and assumes significance only in connection with the other evidence of a relationship between the two diseases.

**2 Geographic Distribution**—Evidence is available to show that rheumatic fever is essentially a disease of the temperate zones. Its incidence diminishes the farther one goes south and it is a relatively rare event in the tropics (Seegal and Seegal,<sup>27</sup> and Coburn<sup>28</sup>). Complete data on the geographic distribution of rheumatoid arthritis are not available but the reports of certain observers are significant. The statistics collected by Clarke<sup>2</sup> from the British Army and the Colonial Medical Services show the same geographic distribution for rheumatoid arthritis as for rheumatic fever. For example, the records of the Federated Malay States show that of 71,208 patients treated in one year no case of rheumatoid arthritis appears. The Straits Settlement Medical Report for the same year presents an analysis of 113,509 patients treated in various tropical countries from Nyassaland to Singapore and in only one instance is the occurrence of rheumatoid arthritis recorded. Clarke analyzed the government returns for two years in thirteen tropical countries and found that out of 1,000,000 inpatients not one was diagnosed as rheumatoid arthritis. Examination of the medical reports of the United Fruit Company, whose estates are largely located in tropical countries, shows little evidence of the occurrence of rheumatoid arthritis. Finally, the reports of several observers in Porto Rico and Jamaica indicate that both rheumatic fever and rheumatoid arthritis are relatively rare events in those islands.

The similar geographic distribution of rheumatic fever and rheumatoid arthritis is a matter of considerable interest. So far as we are aware the only

other example of a specific disease which is equally rare in the tropics is that of scarlet fever. It is true that the incidence of both diphtheria and poliomyelitis diminishes in southern latitudes but both of these diseases have appeared in epidemic form in the tropics. In any case, as Clarke has stated, "the concurrent absence of both rheumatic fever and rheumatoid arthritis in the tropics contrasts so markedly with their concurrent presence in temperate climates that it has left the impression that there may be a causal connection between the two diseases."

3. *Initiating Factors.*—The relation between infection of the upper respiratory tract and the initiation of the rheumatic process is well established. There is general agreement that the relationship is too constant to be a mere chance occurrence. In rheumatoid arthritis the relation is less clear and there are many who will doubt its importance. Nevertheless, conspicuous examples are from time to time observed in which the disease appears to be undoubtedly ushered in by a preceding attack of tonsillitis or sore throat. In our experience

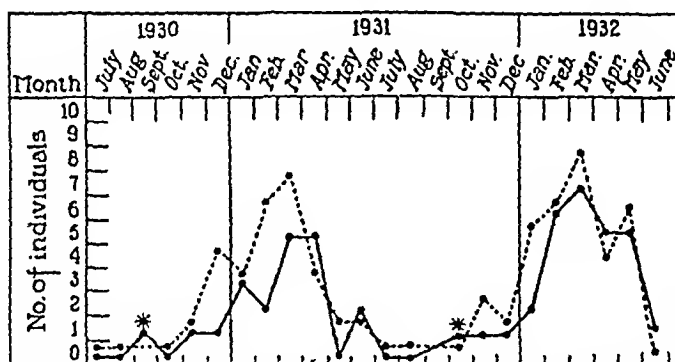


Fig. 1.—Seasonal incidence of hemolytic streptococcus pharyngitis and recurrences of rheumatism among 165 ambulatory rheumatic subjects in New York City. The peaks occurred during the spring months, and were followed by a rapid rise in the incidence of recurrences among ambulatory rheumatic subjects. (After Coburn.)

.....Indicates hemolytic streptococcus pharyngitis.

.....Indicates acute rheumatic fever.

\*Throat cultures not obtained during September, 1930 and 1931. Patients experienced preceding pharyngitis.

such examples are encountered more frequently than is generally supposed. Approximately 20 per cent of cases give a definite history of such infection and in a further 20 per cent a more equivocal history can be obtained. It is our belief that preceding infection of the upper respiratory tract constitutes one of the most important factors in the initiation of the disease rheumatoid arthritis.

4. *Seasonal Incidence.*—In the United States and Canada rheumatic fever exhibits a definite seasonal incidence, reaching a peak in the month of March. This is true not only for the initial attacks but also for subsequent exacerbations. The seasonal incidence of rheumatic recurrences together with the seasonal incidence of hemolytic streptococcal pharyngitis is shown in Fig. 1. In such a chronic and insidious disease as rheumatoid arthritis, it is more difficult to speak of a seasonal incidence. However, a careful study of cases in New York City reveals a seasonal incidence which corresponds closely with that of rheumatic fever. The month of onset in a series of 68 cases in which the time of

onset could be accurately determined is shown in Fig. 2. This similarity in the seasonal incidence of the two diseases constitutes a further link in the chain binding the two conditions together.

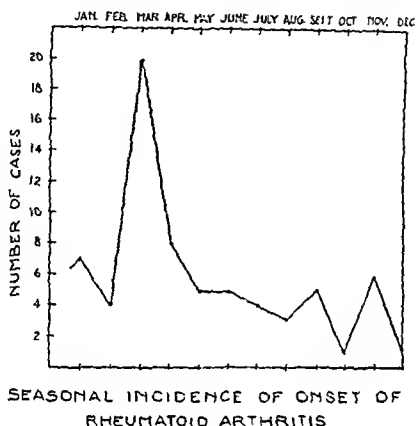


Fig. 2.

## AGE AT ONSET

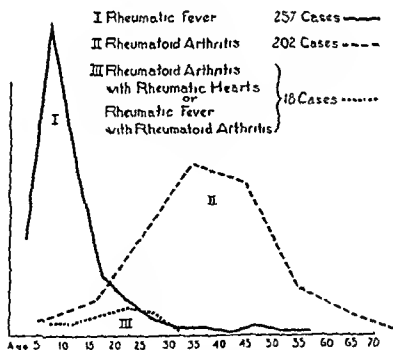


Fig. 3.

5. *Age Incidence and Clinical Manifestations of the Two Diseases in Different Age Periods.*—Rheumatic fever is essentially a disease of childhood while rheumatoid arthritis is predominantly a disease of adult life. The age of onset of 257 cases of rheumatic fever and 202 cases of rheumatoid arthritis is shown in Fig. 3. It will be observed that in nearly 90 per cent of the patients the onset of

rheumatic fever occurs before the age of fifteen while in over 90 per cent of the patients the onset of rheumatoid arthritis occurs after the age of fifteen. More significant, however, is the fact that the clinical manifestations of the two diseases differ widely in different age periods. It is well recognized that rheumatism in childhood presents a different picture from that seen in adult life. As long ago as 1889, 'Headle<sup>29</sup> wrote: "In the rheumatism of early life arthritis is at its minimum; endocarditis, pericarditis, chorea, and subcutaneous nodules at their maximum. As life advances this is gradually reversed; the joint affection becomes prominent, constant and typical of the disease and reaches its maximum; while the other phenomena decline and tend to die out." A summary of the clinical manifestations of rheumatic fever at various age periods is presented in Table I.

TABLE I

CLINICAL MANIFESTATIONS OF RHEUMATIC FEVER AT VARIOUS AGE PERIODS (AFTER COBURN)

INFANCY		CHILDHOOD	
<i>Pancarditis</i>	++++	<i>Pancarditis</i>	++++
Vague symptoms	++	Epistaxis	++++
Pallor	++	Vague symptoms	++++
Chorea	+	Erythemas	+++
etc.		Chorea	+++
		Subcut. nodules	++
		<i>Polyarthritis</i>	++
		etc.	
YOUNG ADULT		ADULT	
<i>Polyarthritis</i>	++++	<i>Chronic arthritis</i>	+++
Epistaxis	++	<i>Polyarthritis</i>	+++
Vague symptoms	++	<i>Pancarditis</i>	±
<i>Pancarditis</i>	++	etc.	
Erythemas	+		
Subcut. nodules	+		
<i>Chronic arthritis</i>	+		
etc.			

The clinical picture of rheumatoid arthritis is more constant in various age periods than that of rheumatic fever, but it also shows significant variations. Attention is called to the occurrence of carditis in classical cases of rheumatoid arthritis, a more frequent event than is generally recognized. In our clinic we have observed 22 examples of this condition and, in a series of 100 consecutive cases, 7 were found to have unequivocal signs of rheumatic heart involvement. It is of special significance that the onset of all these cases occurred in the earlier decades of life, in the majority of instances between the ages of fifteen and twenty-five. Reference to Fig. 3 will show that this is the age period intermediate between that of rheumatic fever and rheumatoid arthritis.

In the minds of many observers the occurrence of typical rheumatoid arthritis in children and the undoubted examples of the development of classical rheumatic fever in the later decades of life may constitute a formidable argument against the hypothesis which is here being presented. In this connection, however, attention should be called to certain facts. In the first place, rheumatoid arthritis in children, or Still's disease, is a relatively rare condition. In a six-year period, during which over 800 cases of rheumatoid arthritis have been observed in

our clinic, only 10 patients under twelve years of age have been seen. In the second place, rheumatoid arthritis in children may at times appear as a fulminating infection accompanied by acute joints, high fever, marked leucocytosis and occasionally pericardial involvement. McCrae<sup>30</sup> described two cases in which pericardial effusion was so marked that tapping was necessary. In three of the cases originally described by Still<sup>31</sup> complete synechia of the pericardium was found at autopsy. It is difficult to offer any explanation for the infrequency of endocardial involvement in Still's disease but, by general consent, pericarditis is one of the most characteristic of all the manifestations of orthodox rheumatic infection. With regard to the development of orthodox rheumatic infection late in life attention is directed to two facts. In the first place, such cases are relatively rare. In the second place, it is exceedingly difficult, even impossible, to be certain that a prior infection has not occurred in the earlier decades of life.

Reference has already been made to the recurrence of rheumatic heart involvement in rheumatoid arthritis. Attention is also directed to the fact that chronic joint changes do develop fairly frequently as a sequel to rheumatic fever. In the German and Scandinavian literature it is customary to refer to these cases by the term "secondary chronic polyarthritis." Freund<sup>3</sup> refers to 110 cases in his own experience while Fischer<sup>21</sup> describes 202 cases. While such an incidence suggests that the disease picture in Europe may be somewhat different from that with which we are familiar in this country, examples of this form of rheumatic disease are not infrequently encountered in a large outdoor clinic. The following statistics from Fischer's clinic on the differentiation of "primary chronic joint rheumatism (rheumatoid arthritis) and "secondary chronic joint rheumatism are also of interest. He states:

- 1 The "primary" form shows its maximum two decades later than the "secondary" form

- 2 The "primary" form leads more frequently (52 per cent) to ankylosis and deformity than the secondary form (37 per cent)

- 3 Cardiac involvement occurs much more frequently (65 per cent) in the secondary form than in the primary form (4 per cent)

Finally, brief mention should be made of those cases in which a differential diagnosis of the two conditions cannot be readily made. It is commonly stated that the disease, rheumatic fever, responds to salicylates, is associated with carditis, and leaves no permanent disability of the joints. Rheumatoid arthritis, on the other hand, does not respond to salicylates, is not associated with carditis and almost invariably proceeds to a permanent disability of the joints. It has already been shown that two of these criteria possess only a relative value and reference will now be made to the third differential point, the different effect of salicylates.

In the minds of many the different effect of salicylates constitutes one of the most fundamental distinctions between rheumatic fever and rheumatoid arthritis. It must be pointed out, however, that salicylates have no effect on the course of events in rheumatic fever except on the exudative manifestations and

as antipyretics. They are quite without effect in the proliferative stage of the disease during which cardiac manifestations are so prone to occur. Furthermore, the effect of salicylates is less dramatic in adults than in children and is occasionally quite disappointing. Finally, salicylates are the most universally used of all drugs in the treatment of rheumatoid arthritis, as well as of rheumatic fever, and, while rarely so dramatic in the former condition, their administration is frequently attended by the greatest symptomatic relief.

It will be admitted by all that, among large groups of rheumatic fever and rheumatoid arthritis patients, many borderline cases occur. They are most commonly observed in the late adolescent and early adult years and are more frequently encountered among ambulatory out-patients than among hospital patients. In our own clinic this type of case is not infrequently seen and defies precise classification in spite of all clinical and laboratory examination.

6. *Pathologic Similarities.*—The most convincing evidence of a relationship between rheumatic fever and rheumatoid arthritis is to be found in the pathologic changes. In the present paper reference will be made only to the similarity of the subcutaneous nodules and the vascular lesions. For a detailed study of other histopathologic changes the reader is referred to the work of Klinge and Grzimek.<sup>23</sup>

Subcutaneous nodules constitute one of the most highly characteristic features of rheumatic fever. In 1881 Barlow and Warner<sup>32</sup> wrote: "Such nodules are in themselves indicative of rheumatism." Cheadle<sup>29</sup> stated that the nodules are "absolutely and solely rheumatic" having, so far as he can judge, "no other origin or connection." Osler<sup>33</sup> expressed the opinion that "their presence may be regarded as a positive indication of rheumatism." Hawthorne<sup>16</sup> in 1900 wrote: "The eruption of fibrous tumors in the subcutaneous tissue . . . is a final and unequivocal proclamation in favor of rheumatism." Coombs<sup>24</sup> has recently stated that the subcutaneous nodule is the most "rheumatic" of all the manifestations of orthodox rheumatic infection. Finally, it has been shown in recent years that the nodules exhibit a highly characteristic histologic structure which is strikingly similar to that of the Aschoff body in the myocardium.

One of us has shown elsewhere that subcutaneous nodules, with a highly characteristic histologic structure, are frequently observed in rheumatoid arthritis.<sup>35</sup> Clinically and pathologically these nodules show a remarkable similarity to those which occur in rheumatic fever. Indeed, the evidence suggests that the nodules in the two conditions represent different phases of the same, fundamental, pathologic process and that such differences as do exist are differences of degree and not of kind. Nodules with a similar histologic appearance have not been described in any other disease. Therefore, as Hawthorne has stated, it is impossible to insist upon the appearance of subcutaneous nodules as a conclusive proof of rheumatic fever and, at the same time, to maintain that rheumatic fever and rheumatoid arthritis are not related diseases. Manifestly one or the other conclusion must be abandoned.

Reference has already been made to the occurrence of cardiac involvement in rheumatoid arthritis. Although of relatively rare occurrence this appears to be of the same nature as that which occurs in rheumatic fever. Unfortunately, it is rarely possible to examine the heart pathologically during the acute stages of rheumatoid arthritis. It is therefore impossible to express any final opinion as to the exact nature of the changes which take place. However, it is of considerable interest that vascular lesions similar to those which occur in rheumatic fever also occur in the vessels of the subcutaneous nodules in rheumatoid arthritis.<sup>1</sup>

**7 Immunologic Findings**—Although the etiology of rheumatic fever has not been finally established a large body of circumstantial evidence has been brought forward by Coburn<sup>28</sup> and others indicating that infection by *Streptococcus hemolyticus* plays a rôle of great importance in the production of the disease. This evidence is for the most part based in clinical bacteriologic and epidemiologic studies. In addition an important immunologic observation has been made by Todd<sup>29</sup> and by Coburn and Paul<sup>30</sup> to the effect that the antistreptolysin titer of the serum increases significantly during a rheumatic attack. This evidence suggests that the rheumatic process is initiated by infection with *Streptococcus hemolyticus*.

In rheumatoid arthritis there is comparatively little clinical bacteriologic or epidemiologic evidence suggesting that *Streptococcus hemolyticus* is concerned in the production of the disease. There is however considerable immunologic evidence of a suggestive nature, though of a different order from that which has been obtained in rheumatic fever. It has been shown that, in the majority of instances the serums of rheumatoid arthritis patients agglutinate hemolytic streptococci in significantly high titers.<sup>31</sup> Agglutination reactions of a similar nature are not observed in any diseases other than those due to hemolytic streptococcal infection. It has furthermore been shown that precipitins for fractions of hemolytic streptococci can be demonstrated in those serums which possess high agglutinating properties.<sup>32</sup> These findings offer suggestive evidence in support of the conception that infection by *Streptococcus hemolyticus* plays a rôle in the production of the disease.

Finally, reference should be made to the fact that the immunologic evidence suggesting hemolytic streptococcal infection is of a different nature in the two diseases. In rheumatic fever the serums show an increased antistreptolysin content but do not contain agglutinins in significantly high titers. In rheumatoid arthritis serums on the other hand significant agglutinins are present but the antistreptolysin content is not elevated, except in early and active cases.

In the present state of knowledge it is difficult to evaluate the significance of these immunologic findings in the two diseases. It is possible that they may represent different responses to infection by the same agent but no final opinion may be expressed until the etiology of both diseases has been definitely established.

#### DISCUSSION

The relationship between rheumatic fever and rheumatoid arthritis is, at the present time, of greater theoretical than practical importance. For clinical



purposes it is important that the two should be differentiated whenever possible for, in typical cases, each presents its own symptoms, each demands its own therapeutic management and each requires its own prognosis. For theoretical reasons, however, a clearer understanding of the nature of the relationship of the two diseases is of great importance and may contribute much to our knowledge of both conditions.

Evidence has been presented to show that rheumatic fever and rheumatoid arthritis are intimately related and possibly different manifestations of the same pathologic process. Of particular significance is the clinical and anatomical evidence obtained from a study of "atypical" and "borderline" cases. The clinical evidence suggests that the two form a continuous sequence of one disease process with different expressions in each individual phase. These different expressions appear to be in large measure determined by the age of the patient but undoubtedly other factors, such as individual host susceptibility, are also of importance. The pathologic evidence, representing a difference in degree rather than in kind, strongly suggests that the two represent different responses to the same, or closely related, etiologic agents.

A final understanding of the relationship between rheumatic fever and rheumatoid arthritis will not be possible until the etiology of both diseases has been definitely established. At the present time there is a certain amount of evidence suggesting that infection by *Streptococcus hemolyticus* plays a rôle in the production of both diseases. However, this evidence is as yet far from complete and, even if it could be established that both diseases were due to the same agent, it would not prove their identity. The situation would then be analogous to that which exists with syphilis and yaws. Here the etiology of both diseases is definitely known, yet the relationship between the two is still a matter of controversy. It is obviously more difficult, with the knowledge at present available, to arrive at a complete understanding of the relationship between rheumatic fever and rheumatoid arthritis.

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## DISCUSSION

DR CURRIER McEWEN, NEW YORK, N Y.—A few years ago at the suggestion of Dr Swift, we studied supravitally the cells of the nodule subcutaneous nodules, to obtain further information about the nature of the typical cells of rheumatic granulomas. The appearance of these cells when exposed to Janus green and neutral red was found to be different from that of cells of certain other granulomas. It seemed useful to conduct a similar study of the subcutaneous nodules of rheumatoid arthritis. In nodules from eight such cases we have been able to examine the living cells and have found them in all essentials the same as those of the subcutaneous nodules of rheumatic fever. Similar studies of granulation tissue removed from joints at operation showed the cells to have the same characteristics. This is in marked contrast to syphilitic and tuberculous granulation tissue. Thus it is seen that not only is the general microscopic appearance of the granuloma of rheumatic fever and rheumatoid arthritis similar, but also the individual cells of these lesions are the same. This does not by any means prove the identity of the two diseases, but it is one more link in the chain of evidence connecting the two.

DR JOHN R PAUL, NEW HAVEN, CONN.—Drs Dawson and Tyson deserve credit for emphasizing with such vigor a relationship between these diseases. Many have hinted at their similarity, but few have insisted that they are both the same disease. It seems to be essentially a question of definition. As it is difficult or impossible to define rheumatic fever, it is equally difficult to insist that another disease picture actually is rheumatic fever.

Drs Dawson and Tyson suggest that the difference between the two conditions is a question of virulence of the organism or of the susceptibility of the host. It seems to me that the susceptibility of the host should be considered first, in that there are certainly few diseases in which the symptomatology is conditioned by age more than it is in rheumatic fever, resembling tuberculosis in that respect. The presence of different types of cases of rheumatic fever and rheumatoid arthritis within the same family is perhaps an example of this age susceptibility. We recently saw a family in which a child of four years developed acute rheumatic carditis, a child of nine, subacute rheumatic fever with joint involvement, and just about the same time the mother developed rheumatoid arthritis.

It is one thing to admit that there is probably a relationship between these two conditions, but it is another thing to have to act upon the suggestion. We have had this decision brought to us very seriously in our work on the epidemiology of rheumatic fever.

among families and communities. In certain communities we have been struck by the high incidence of rheumatic fever. We have also found a high incidence of cases of rheumatoid arthritis in these same communities. For our own practical purposes we have, in some instances, almost been forced to consider these two clinical pictures as manifestations of one disease.

DR. M. J. SHAPIRO, MINNEAPOLIS, MINN.—Dr. Dawson has made a good case in favor of the relationship between rheumatic fever and rheumatoid arthritis. However, in following a number of cases of rheumatic fever over a period of years, I have never observed a single patient with rheumatic fever develop chronic arthritis. Many of my patients have reached the period between twenty and thirty years of age when they would be expected to develop rheumatoid arthritis. If these two diseases are closely related it seems to me that I should have observed an occasional case of rheumatic fever develop into rheumatoid arthritis. I have seen four instances when patients in the early teens have developed long-continued swelling about various joints which looked much like rheumatoid arthritis but in each instance the joints did finally clear up completely with no change therein as evidenced by x-ray examination. Those of us who are studying rheumatic fever from its inception should be able to answer this question if we live long enough.

DR. HOMER F. SWIFT, NEW YORK, N. Y.—We have heard much about the age incidence in the development of these diseases, but have possibly neglected another important temporal factor, the period during which the patient may have been exposed to conditions, either infectious or environmental, favorable for the development of the particular type of rheumatism from which he may be suffering. This idea arises from an interesting observation of Dr. Boas a few years ago, that adult Porto Ricans who had recently come to New York from Porto Rico often had the type of rheumatic fever we are accustomed to see in children who have resided in New York all their lives. It is probable that the lack of rheumatic fever in Porto Rico has resulted in these people not developing any resistance to the disease, so that when they suffer their first attack their tissues are in a condition somewhat comparable to that of children.

DR. J. A. KEY, ST. LOUIS, MO.—It is interesting to study two diseases that are so different. I do not know much about rheumatic fever, but I do feel that it is quite dangerous to assume that two diseases are identical or closely related because similar nodules are present in each. Personally, it has been my impression that rheumatoid arthritis was not familial and that hypertrophic arthritis might be familial. I hope that Drs. Dawson and Tyson will continue their studies and later give us a paper on the differences between rheumatoid arthritis and rheumatic fever.

DR. R. L. CECIL, NEW YORK, N. Y.—Dr. Dawson's ideas are intriguing. It has occurred to me, however, that there is one stumblingblock in the way of accepting them, namely, the occurrence of Still's disease in children. If the differences between the two diseases are dependent largely on age, it would be hard to explain Still's disease, a condition now generally looked upon as rheumatoid arthritis in childhood.

DR. DAWSON (closing).—We appreciate that rheumatic fever and rheumatoid arthritis in their classical forms are distinct clinical entities, and we believe that it is important that they should be distinguished clinically. However, we do insist that the two disease pictures overlap and that it is frequently impossible to separate them.

We do not feel that the occurrence of Still's disease, which we regard simply as rheumatoid arthritis in children, constitutes a serious obstacle to our argument. After all, cases of Still's disease are relatively rare and these patients not infrequently show evidence of cardiac involvement, usually pericardial. On the other hand, one occasionally sees cases of orthodox rheumatic fever late in life; but, again, such cases are most unusual compared with the incidence in childhood.

Regarding the development of chronic joint changes following orthodox rheumatic infection, Dr. Shapiro does not encounter them because he is working exclusively with children.

We are dealing with an older group of patients and such cases not infrequently come to our notice. Furthermore, we feel that there may be a difference in the clinical picture of these two diseases in different parts of the world. For example, Freund in Vienna reports 110 cases of "secondary chronic polyarthritis" and Fischer in Germany reports 202 cases in his own personal experience.

A plausible explanation for the different conceptions held by different clinicians regarding the relationship of the two diseases may be found in the type of cases studied. We have primarily been interested in a large group of ambulatory patients. In our material borderline cases are encountered much more frequently than is generally supposed.

## GEOGRAPHIC DISTRIBUTION OF RHEUMATIC FEVER AND RHEUMATIC HEART DISEASE IN THE UNITED STATES\*

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WHEN it was suggested that a summary of our knowledge of the regional distribution of rheumatic fever and rheumatic heart disease in this country would be timely, I was reminded of a remark by Poynton under somewhat similar circumstances in England some years ago when he said, "I am in the position of one who has already overwritten himself on the subject (rheumatic fever), and we know that knowledge advances much more slowly than the writing of papers." So it is with apology for repetition that I have gathered together in review the data showing the variable geographic distribution of both rheumatic fever and rheumatic heart disease in the United States. It is hoped that the restatement of these surveys may stimulate others to appraise more accurately the incidence of this disease in their own regions. By way of emphasizing the influence of geographic location on the clinical incidence of rheumatic fever and rheumatic heart disease, my studies on this score in southern Florida are included.

Compilation of hospital statistics from various cities by Faulkner and White<sup>1</sup> and Harrison and Levine<sup>2</sup> in 1924 indicated that the incidence of rheumatic fever is much lower in the southern than in the northern part of the United States. In 1926, Wood, Jones and Kimbrough<sup>3</sup> showed by comparative analysis of hospital records that rheumatic heart disease is half as common in Virginia as in Massachusetts. It was further shown by the collection of hospital data of Seegal and Seegal<sup>4</sup> in 1927 that although the incidence of rheumatic fever anywhere varies with the years, yet for a given period of years the incidence in southern cities is markedly lower than in northern cities of this country. It should be noted that these authors, recognizing that data compiled from questionnaires filled out by hospital record clerks might be inaccurate, cautioned against too much reliance being placed on their rates.

Other studies of the incidence of rheumatic fever or rheumatic heart disease in various localities have been recorded and are included in Tables I and II. In considering this data it is necessary to keep in mind just what concept each author had regarding the inclusiveness of the term "rheumatic fever." In general the condition may be said to include cases with and without arthritis, rheumatic carditis whether acute, subacute or "smoldering," and chorea. Chronic, inactive cases of rheumatic heart disease were not usually classed as rheumatic fever. Any departure from this usage of the term will be noted in the tables, but two instances merit special comment. In 1931 Longcope<sup>5</sup> in discussing the variations in the manifestations of rheumatic

\*Read at the Second Annual Meeting of The American Association for the Study and Control of Rheumatic Diseases and the Fourth Conference on Rheumatic Diseases, June 10, 1935, Atlantic City, N. J.

fever in relation to climate, stated that in Baltimore the incidence of rheumatic fever admissions to adult medical wards of Johns Hopkins Hospital was 13 per cent which is equivalent to the rate given for a similar hospital in Boston. This is surprising until one discovers that Longcope counted cases of quiescent or inactive rheumatic heart disease as examples of rheumatic fever. The discrepancy becomes apparent on comparing the autopsy statistics given by the same author, which indicate that rheumatic heart disease is encountered at autopsy in Baltimore with less than half the frequency found in Boston. This author did emphasize, however, that the arthritic manifestations of rheumatic fever are less outspoken in that locality, compared with cities farther north, and that it is essentially a disease of the heart of a rather insidious type. Likewise in 1933, McLean<sup>6</sup> in computing the admission rate

TABLE I

ADAPTED FROM PUBLISHED DATA REGARDING HOSPITAL INCIDENCE OF RHEUMATIC FEVER IN THE UNITED STATES

LOCATION OF HOSPITAL	LAT °	YEARS IN CLINIC	MED ADM W/ H FEVER OF CHOREA	REPORTED BY
Spokane, Wash	47	1920	30	S & S†
Portland, Ore	45	1920	1245	S & S
Portland, Ore	45	"	01	Coffen†
Boston	42	1914-20	18	H & L
Boston	42	1915	10	F & W
Cleveland	41	1900-20	2000	S & S
Iowa City	41	1923	4	F & W
Omaha	41	192	0	F & W
New York	40	191	2060	S & S
New York	40	1917-21	1138*	Davis†
Philadelphia	39	1915-2	012	F & W
Baltimore	39	1915-2	0	H & L
Baltimore	39	1915-20	104	Longcope†
Denver	39	1924-25	1001	S & S
St Louis	38	1900-2	04	F & W
St Louis	38	1916-2	10	H & L
University Va	38	1920-2	15	H W & D
San Francisco	37	1900-25	1276	S & S
San Francisco	37	1919-20	75	F & W
Norfolk, Va	36	1905-20	015	H W & D
Oklahoma City	35	1922-23	0002	S & S
Memphis	35	1920-23	0105	S & S
Los Angeles	34	1921-24	04	F & W
Augusta Ga	33	1918-21	008	F & W
Birmingham	33	1917-2	0005	S & S
Birmingham	33	1922-32	181	McLean <sup>6</sup>
San Diego	32	1920	013	S & S
Charleston	32	1920-23	0102	S & S
Dallas	32	1919-23	000	S & S
New Orleans	29	1915-20	0110	S & S
New Orleans	29	1916-20	0	H & L
Galveston	29	1915-25	0002	S & S
Galveston	29	1915-23	08*	H & L
Tampa	27	?	04	B & C
Miami	25	1930-34	013	Nichols†

\*Cases of chorea not included

†Includes only cases with arthritis

‡Includes also chronic inactive rheumatic heart disease

§My own figures added for comparison

§S & S Seegal and Seegal; H & L Harrison and Levine; F & W Faulkner and White; H W & D Hart Wood and Daughton; B & C Blitzer and Cooke†

of rheumatic fever at the Children's Hospital in Birmingham included all instances of *chronic* or *inactive* rheumatic heart disease, with the result that the rate obtained of 1.8 per cent is considerably higher than it would be had the usual clinical concept been followed. There is no doubt, however, that the incidence of rheumatic fever in Birmingham is relatively high compared with some sections of the South, though unfortunately no autopsy statistics were given. McLean also showed the infrequency of arthritis as a manifestation of rheumatic fever in Birmingham and stressed that there, also, rheumatic fever is primarily a disease of the heart.

Another factor possibly detracting from the reliability of the comparative data in Tables I and II is the varying alertness of the attending physicians making the diagnosis of rheumatic fever in each instance, or judging whether a given case of heart disease is of rheumatic etiology. In children in particular it is commonly admitted that the early stage of heart disease is not an easy subject. The variable picture of the rheumatic state has been amply portrayed in clear fashion by Coburn,<sup>7</sup> and in a recent monograph dealing with the etiology of rheumatic heart disease, Paul<sup>8</sup> has stressed the difficulty of making accurate etiologic studies of a disease so variable in its presenting symptoms.

TABLE II

REPORTED FREQUENCY OF RHEUMATIC TYPE OF HEART DISEASE AMONG HOSPITALIZED  
"CARDIAC" PATIENTS IN VARIOUS CITIES

HOSPITAL LOCATION	LAT. N.°	YEARS	% OF RHEUMATIC HEART DISEASE	REPORTED BY
Portland, Ore.	45	1929	10.1	Coffen <sup>14</sup>
Boston	42	1924	39.8	Wood, et al. <sup>3</sup>
Chicago	41	1932-33	16.7	Flaxman <sup>18</sup>
Salt Lake City	40	1927-29	40.4	Viko <sup>19</sup>
Louisville	38	1928-33	8.0	Simmons <sup>20</sup>
University, Va.	38	1923-26	21.9	Wood, et al.
Washington, D. C.	38	?	6.0	Gaeger and Dunn <sup>21</sup>
Nashville	36	1930-31	10.5	Laws <sup>22</sup>
Galveston	29	1920-26	7.3	Stone and Vauzant <sup>23</sup>
Miami	25	1931-34	19.0*	Nichol

\*Only 2 per cent was acquired in Florida. My own figures added for comparison.

In spite of these difficulties, after making allowance for the probable errors in the collected data and the difference in clinical concepts, it seems apparent on reviewing the data, that, on the whole, clinically perceptible rheumatic fever and rheumatic heart disease are much less common in southern than in northern regions of the United States. Nevertheless, a comparison of the frequency with which rheumatic heart disease is encountered at autopsy in various sections of the country obviously should give the most accurate index of the influence of geographic location and climate on the occurrence of rheumatic fever. Some of the autopsy data given by Harrison and Levine<sup>2</sup> and a few other reports of the incidence of rheumatic heart disease found at autopsy in certain regions have been combined in Table III. My own figures for southern Florida have been added for comparison. It is only fair to point out that in some southern cities autopsy statistics may give rise to an

exaggerated notion of the incidence of rheumatic fever, since the winter resort regions are invaded during the winter months by many individuals from the North with rheumatic heart disease some of whom eventually die while in the South and make up a fair share of cases arriving at necropsy. It will be seen

TABLE III  
REPORTED FREQUENCY OF RHEUMATIC HEART DISEASE FOUND AT AUTOPSY  
IN VARIOUS CITIES

HOSPITAL LOCATION	LAT N°	YRS	% OF RHEUMATIC HEART DISEASE	REPORTED BY
Boston	42	1913-23	1.0	Harrison and Levine*
Cincinnati	39	1927-30	2.0	Glazer <sup>21</sup>
Baltimore	39	1925-30	1.6	Longcope <sup>22</sup>
Oklahoma City	35	1913-24	0.0	Harrison and Levine
New Orleans	29	1910-1	0.2	Harrison and Levine
Miami	25	1911-4	4.1	Nichol (present report)

\*Actually only 0.5 per cent if a low rate made for migration of patients

the available data indicate that the rate of rheumatic heart disease found at autopsy in southern cities is much less than in northern cities. It has been suggested<sup>23</sup> that since these studies are based on gross pathologic findings, such as the presence of mitral stenosis or rheumatic pericarditis that further studies should be made including microscopic examination of standard sections of the heart tissues to make sure that some grades of rheumatic carditis have not been overlooked.

#### INCIDENCE IN SOUTHERN FLORIDA

A more striking portrayal of the way geographic location and climate influence the distribution of rheumatic fever and rheumatic heart disease in this country is seen on comparing my own studies in southern Florida (25° latitude N) with the figures from Boston (43° latitude N). Having made further inquiry since my previous reports<sup>10</sup> on this subject, I have combined the data for the five year period from 1930 to 1934 inclusive, showing the incidence of rheumatic fever and rheumatic heart disease at Jackson Memorial Hospital, Miami, an institution admitting patients of all descriptions. Previously published data for the years prior to 1930 are not included, as it is only since that date that practically all patients admitted to the hospital wards with rheumatic fever or rheumatic heart disease have been examined by the author. It is felt that this close acquaintanceship with the patients making up this data lends considerable weight to its importance as all questionable instances of rheumatic infection were carefully studied in order properly to classify them.

During the five years there were only 11 cases of rheumatic fever or carditis, acute, subacute or "smouldering," and no cases of chorea, found among 8,287 medical admissions, including children, giving an admission rate of 0.13 per cent (Table IV). This is in sharp contrast to the admission rate during the same period at the Peter Bent Brigham Hospital, Boston, of 1.4 per



cent, as determined by Levine<sup>11</sup> who found 137 cases of rheumatic fever, chorea or carditis, acute or "smouldering," among 9,817 medical admissions. (The incidence would have very likely been even higher if children under twelve years were admitted to this hospital.)

TABLE IV

COMPARISON OF HOSPITAL MEDICAL ADMISSIONS FOR RHEUMATIC FEVER IN BOSTON AND MIAMI DURING 1930-34 INCLUSIVE

HOSPITAL	CITY	NO. MED. ADM.	NO. CASES RH. FEVER, RH. CARDITIS OR CHOREA
Peter Bent Brigham	Boston	9,817	137* or 1.4 %
Jackson Memorial	Miami	8,287	11† or 0.13%

\*Twenty-three cases of chorea.

†No chorea; only 3 cases with arthritis.

TABLE V

INCIDENCE OF RHEUMATIC HEART DISEASE IN MIAMI AS DETERMINED FOR THE YEARS 1931-34 INCLUSIVE

NO. "CARDIAC" CASES OF ALL TYPES FROM		NO. CASES WITH RHEUM. HEART DISEASE	NO. CASES RHEUM. HEART DISEASE ACQUIRED WHILE IN MIAMI
Jackson M. Hosp.	561	106 or 19.0%	11 or 2.0%
Office practice	408	68 or 16.6%	2
Total	969	174 or 17.9%	13 or 1.3%

\*Part of these cases are included in a previous communication.<sup>10</sup>

In view of this extremely low incidence of active rheumatic infection in Miami, it would be expected that in a series of cardiac patients hospitalized, the number classed as being of rheumatic origin would be correspondingly small. It is seen in Table V, however, that during the years from 1931 to 1934 inclusive there were 106 instances of rheumatic heart disease among 561 patients with cardiac disorders in the hospital. Although the percentage obtained (19.0 per cent) is only half that reported in some northern hospitals, it exceeds the ratio given for some northern points, as was seen in Table II. This discrepancy might indicate that many instances of *active* rheumatic carditis were overlooked in the hospital wards, but a study of the clinical histories of the patients classed as rheumatic heart disease reveals that *all* had acquired their structural cardiac damage before migrating South, except for those described above with *active* rheumatic infection. Thus only 2 per cent approximately of the total group of patients with heart disease should be classed as having rheumatic heart disease originating in Florida.

The frequency with which rheumatic heart disease is encountered in office practice in southern Florida is also illustrated in Table V. Of 408 cardiac patients examined in the period from 1931 to 1934 inclusive, 16.5 per cent or 68 were classed as being of rheumatic etiology, but with *one* exception all had acquired rheumatic disease before leaving the North, and in only *one* instance was there clinical evidence of reactivation of a "healed" rheumatic carditis among the 68 patients during their stay in the South. On combining the hospital and office data, it is seen that there were 174 instances of rheumatic heart disease among 969 cardiac patients (17.9 per cent). But only

13 patients or 13 per cent of the total group acquired rheumatic heart disease during their life or residence in Florida. In contrast to this, it has been shown by White and Jones<sup>12</sup> that in New England (Boston) among 3,000 patients with heart disease encountered in hospital, clinic, and private practice, 31.9 per cent were classed as being of rheumatic etiology.

On considering the available pathologic data, it was found that among 401 autopsies performed during the past four years at Jackson Memorial Hospital, there were 89 instances in which death was due primarily to heart disease, and of these 16 or 4 per cent gave anatomical evidence of *rheumatic* heart disease. But again on inspecting the clinical histories, it is shown that in fourteen instances the rheumatic pathologic changes had been noted in the North, and that in only two instances was the rheumatic infection acquired in the South. Thus, actually, only 0.5 per cent of the autopsies performed during this period showed rheumatic heart disease originating in Florida.

#### COMMENT

Many physicians have questioned the actual rareness of rheumatic fever in the South. As to the validity of these doubts for some sections of the South, I cannot vouch, but ten years of observation of the scene in southern Florida has convinced me that as far as this section of the South is concerned even the very mild or smouldering form of rheumatic carditis is quite uncommon while the obvious cases with arthritis or chorea are rare indeed. During the past five years in particular I have watched for the subclinical forms of rheumatic fever, which, it was claimed following my first report, probably escaped clinical notice. In my opinion there is a little ground for the feeling expressed editorially recently by Hatch and others<sup>13</sup> that it might be better to have rheumatic fever in the North where it is recognized early due to its more acute form, than in the southern states where the diagnosis might be missed, owing to an insidious onset, thus favoring more damage to the heart of the victim, who would go untreated for a longer period. If this were the case a general hospital in southern Florida should provide a far greater number of cases of rheumatic heart disease than it does, as seen above.

#### SUMMARY

A review of available data indicates that there is a definite inequality in the distribution of rheumatic fever and rheumatic heart disease in the United States, the amount being much less in the southern states. The influence of geographic location on the clinical incidence of rheumatic fever and rheumatic heart disease is emphasized by contrasting the findings in southern Florida and New England. During the past five years, in spite of a careful search for subclinical cases, the admission rate of rheumatic fever, rheumatic carditis or chorea in a general hospital in Miami was only one tenth the rate in Boston during the same period.

Only 13 per cent of "cardiac" patients found in Miami both in hospital and office practice had rheumatic heart disease determined clinically to have been acquired during life or residence in the South, compared to a recent estimate that 31.9 per cent of "cardiac" patients encountered in New England were of rheumatic type.

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That the incidence of the disease may vary in the same latitude is indicated by the report of Hart and Wood, who found an incidence of rheumatic fever at the University of Virginia Hospital in 1934 of 0.48 per cent of the total hospital admissions, while in the same year in the same latitude at Norfolk, Virginia the incidence was 0.15 per cent of total admissions.

There are certain rank discrepancies in the reports from over the world on the occurrence of this disease which require verification. Charl states he has seen no rheumatic fever or mitral disease in the Malay States or in the British Army in Southern India, while Church reports a high incidence of rheumatic fever in Ceylon. Menje reported a high incidence in Tahiti, whereas in the Sanssou Islands a very short distance away in the same latitude there is none. A verification of these and other discrepancies in the literature may throw a considerable amount of light on the possibilities in the etiology of this disease.

During the past five years the House of the Children's Hospital has sent each year a small group of children with rheumatic fever to the St. Francis Hospital in Miami Beach, Florida, in an attempt to evaluate if possible the effect of a subtropical climate on the course of the disease. During the first five years twenty-three children were sent. In summary of this study it may be said that it would seem with resulting death do occur in this climate, but in general the patients do better and the convalescences are shorter lived than in the North. It should be stressed however that the complications of rheumatic fever do occur in rheumatic patients while they are ill.

DR CURRIER McEWEN, New York N. Y. As a result of the statement that rheumatic fever does improve in the tropics I feel that the same is true of atrophic arthritis.

DR JOHN R. PAUL, New Haven, Conn. I feel that we soon begin to get some idea of the general prevalence of rheumatic fever in this country. I doubt if nationwide data can be satisfactorily obtained from the statistics on list but a more satisfactory method is the determination of the prevalence of heart disease among school children in different parts of the country.

DR M. H. DAWSON, New York N. Y. While I am a student in a hospital in Jamaica, B. W. I., Dr. Dochez was shown a case of rheumatic fever. He remarked that he had understood that rheumatic fever was a very rare disease in the tropics. The attending physician replied, "Oh no we see cases from time to time, but there is a very curious thing about them, they all come from up in the hills," where, of course, the climate is not truly tropical.

DR S. A. LEVINE, Boston, Mass.—I would like to comment on the difference in incidence of rheumatic fever in different parts of the country. Physicians down South call rheumatic fever arthritis and so term it in their records. They say that we in the North see more rheumatic fever. In a large general hospital rheumatic heart disease must be seen if it exists in the community for it is eventually fatal. In a hospital of 200 beds in the course of ten years there will be a certain number of patients with rheumatic mitral stenosis. At death it will be recognized by the pathologist no matter what it was called in life. At the Charity Hospital in New Orleans the incidence of mitral stenosis was one twentieth of that which we found at the Brigham Hospital in Boston. This was conclusive evidence to us that either rheumatic fever was markedly less common in New Orleans than it was in Boston or that rheumatic fever occurring down there does not affect the heart like it does in Boston.

DR NICHOL (closing)—In reply to Dr. McEwen's question, I have no accurate data on the geographic incidence of atrophic arthritis. From an experience of ten years in southern Florida I would state that a patient with atrophic arthritis does not improve at the same rate that one with rheumatic heart disease does in the same period of time. I was asked to prepare some statistics regarding the incidence of atrophic arthritis, but I did not attempt to do so, because statistics not given from one's own experience have very little value.

No one should overlook Dr. Paul's excellent studies on school children. Three years ago I made a survey of the school children in Miami. The difficulties are almost insurmountable for a "one man job." On comparing 1,500 school children born and reared in Miami with 1,500 school children who had migrated to Miami I found that the incidence of rheumatic heart disease was four times greater in the "migrated" group than in the native group. However, some of those children had been sent to Florida because of a previously established rheumatic disease.

# AN OUTLINE OF STUDIES RELATING TO VITAMIN C DEFICIENCY IN RHEUMATIC FEVER\*

JAMES F. RINEHART, M.D., SAN FRANCISCO, CALIF.

**I**N PREVIOUS reports<sup>1</sup> experimental data have been presented indicating that under the combined influence of vitamin C deficiency and infection guinea pigs developed with considerable frequency, endocardial, myocardial and articular lesions bearing considerable resemblance to those of rheumatic fever. On the basis of these observations the concept was advanced that rheumatic fever may be the result of the combined influence of vitamin C deficiency and infection. Further experimental studies have confirmed and extended the original findings. It is the purpose of this communication to review the broad experimental basis for the concept to consider certain epidemiologic and clinical data bearing on the problem and finally to summarize the basic pathology of rheumatic fever and relate it to the thesis advanced.

## EXPERIMENTAL METHODS

As detail of experimental methods has been previously published,<sup>1</sup> a brief outline will serve for the present communication. Guinea pigs were maintained on a basic diet containing all other food factors but devoid of vitamin C. In different experiments, varying grades of deficiency were maintained by regulating the vitamin C supplement which was given in the form of orange juice. The infecting agents in the majority of instances were hemolytic streptococci derived from spontaneous cervical lymphadenitis which is a relatively common infection in guinea pigs. This use of natural pathogens for the species in all of the experiments is believed to be of considerable importance. It should be noted that there was definite variability in virulence of different strains of hemolytic streptococci used. The most striking lesions occurred in experiments where the virulence of the organism was great. Infection was transferred by intracutaneous inoculation of pure broth cultures of the organism. This resulted in a localized infection of the skin and regional lymph nodes. Occasionally but not commonly metastatic abscesses occurred in distant sites. It should be pointed out that almost uniformly the local infection in the deficient animals was more intense, less well localized, and less readily healed, than in the control animals receiving adequate vitamin C supplements. While the most extensive observations have been made with the hemolytic streptococcus as the infecting agent and the most striking lesions have been observed with this organism, a few other bacteria including a gamma type streptococcus, *B. aertrycke*, and *B. bronchisepticus* have been used. Studies with these organisms have not been extensive, however, the occasional development of "rheumatic type" lesions, in which infection with these agents has been superimposed on the scorbutic state, suggests that the bacterial factor is not specific.

\*From the Department of Pathology, University of California Medical School.  
Read at the Second Annual Meeting of The American Association for the Study and Control of Rheumatic Diseases and the Fourth Conference on Rheumatic Diseases, June 10, 1935, Atlantic City, N. J.

An *active* or *virulent* infection would appear to be of more importance than the exact organism. This phase of the problem, however, requires further study.

#### EXPERIMENTAL FINDINGS

In each of a rather extensive series of experiments, the effects of the uncomplicated deficiency, the deficiency with superimposed infection, and the infection in the adequately nourished animal were studied. *The various infecting organisms in the presence of adequate nutrition, did not produce rheumatic type lesions.*

Inasmuch as the anatomic changes in the heart and articular tissues have previously been described and illustrated,<sup>1, 2</sup> a brief review of the findings will suffice for the present communication. Subsequent studies have simply confirmed and extended the previous reports.

*Heart Valves.*—In uncomplicated vitamin C deficiency, atrophic and degenerative changes develop in the collagenous stroma of the heart valves. Rarely mild proliferative reactions accompany this degeneration. In vitamin C deficiency with superimposed infection, lesions of a combined degenerative and proliferative nature develop with considerable frequency. These lesions vary considerably and are sometimes striking in character and bear close resemblance to the early rheumatic endocarditis. Ribbert,<sup>3</sup> Clawson,<sup>4</sup> Poynton and Schlesinger,<sup>5</sup> and others have clearly shown that the rheumatic vegetations develop within the valve substance, due to subendothelial proliferation of cells which is most prominent at the line of closure. Small amounts of true fibrin may merge with the cells in the superficial zone. This is essentially the nature of the experimental lesions as well.

*Heart Muscle.*—The pathologic changes in the heart muscle are less striking than the valvulitis. However, focal proliferative reactions are not infrequently observed. These may be found beneath the mural endocardium, in the pericardium and in the muscle. Some of the most significant reactions develop near the angle of attachment of the mitral valve. The reason, perhaps, that the lesions occur at this site is that there is here, as in the valve proper, a significant amount of intercellular substance (collagen) subject to undue stress and injury which forms the nidus for the proliferative focus. The connective tissue about the coronary vessel branches in the guinea pig heart is insignificant compared with that in the human heart.

The experimental proliferative foci, although not identical, are considered of a basically similar sort to the Aschoff reaction. The cells surround altered collagen and the nuclear and cytoplasmic character of the reacting cells correspond very closely to those composing the Aschoff body.

*The Joints.*—An early manifestation of vitamin C deficiency in the guinea pig is an arthropathy characterized by pain and swelling. Certain infections superadded to the deficiency accelerate and augment this arthropathy. The same infections in the presence of adequate nutrition do not affect the joints. Pathologic observations on the joints in rheumatic fever are not abundant. The most exhaustive study of the articular changes in rheumatic fever is afforded by the studies of Klinge,<sup>6</sup> and Klinge and Grzimek.<sup>7</sup> These authors find the lesions to be characterized by "fibrinoid" degeneration and granulom-

atous reactions in the capsular and periarticular tissues, mild hyperplasia of the synovial membrane with "fibroid" degeneration in the synovial and subsynovial tissues and the presence of hyaline fibrous material free in the joint space. It is noteworthy that just such changes occur in the experimental animals.<sup>7</sup>

Much evidence has gradually accrued relating rheumatic fever and atrophic (rheumatoid) arthritis. Klinge and Gizmek<sup>7</sup> hold that although acute or subacute rheumatic fever and atrophic (rheumatoid) arthritis may usually be differentiated, both disease pictures are so intimately related in both joint and general pathology that a "rheumatic" basis may be assigned to both. Dawson and Tyson<sup>8</sup> have recently summarized a rather convincing mass of data indicating a relationship between the two diseases. It is perhaps not without significance that in the experimental studies under consideration, no sharp line can be drawn between a disease picture resembling rheumatic fever and one characterized by a chronic deforming arthritis. Subacute or chronic vitamin C deficiency in the guinea pig produces a painful deforming arthropathy with manifold similarities to atrophic (rheumatoid) arthritis. These include synovial proliferation, intra-articular pynus formation and periarticular fibrous tissue overgrowth. In certain instances, superimposed infection accelerates and accentuates the pathologic process.<sup>9</sup> The general atrophic changes found in atrophic (rheumatoid) arthritis involving the bony skeleton, muscle, and skin are also observed.

*Subcutaneous Nodules*—One feature common to both rheumatic fever and atrophic (rheumatoid) arthritis is the subcutaneous rheumatic nodule. Dawson<sup>10</sup> particularly has drawn attention to this lesion and finds the early pathologic histology in both conditions to be essentially the same. The lesion is characterized by hyaline streaks of fibrin surrounded by a reactive overgrowth of connective tissue cells. At times the fibrin is intimately intermingled with collagen. In the experimental animals particularly those subjected to the more chronic deficiency, such nodules have been seen about joints with considerable frequency.

#### SUMMARY

It will be seen then that the concept that vitamin C deficiency may be a contributory factor in the etiology of rheumatic fever lies upon a broad experimental base. With vitamin C deficiency and infection combined lesions comparable to those of rheumatic fever are found not only in the heart valves and muscle, but also in the joints. Subcutaneous nodules serve to complete the pathologic similarity. No claim is made for the identity of the experimental lesions to those of the human disease. They are, however, thought to be *fundamentally similar*.

#### EPIDEMIOLOGIC CONSIDERATIONS

Malnutrition has commonly been observed in rheumatic fever. The geographic distribution, the dominant urban incidence, the high incidence in winter and spring and the frequent familial occurrence, might be explained upon the basis of greater liability to infection. On the other hand, these



features are entirely in accord with the operation of a factor of vitamin C deficiency. One epidemiologic peculiarity that particularly suggests an environmental factor other than infection in the genesis of rheumatic fever is the intimate relationship of the disease to poverty. Glover<sup>11</sup> believes that the true incidence of rheumatic fever in England is directly proportional to the degree of poverty and estimates that the occurrence of acute rheumatism is twenty or even thirty times as great in the poor as in the well-to-do. It would appear to me that a disease with such an amazingly high incidence in poor people could not be explained on the basis of a specific infecting factor or on the basis of recurrent nonspecific infection alone. Some fundamental environmental or nutritional influence would seem to lie in the background.

#### CLINICAL OBSERVATIONS

During the past eighteen months a clinical approach to the problem has been made in association with Dr. Amos Christie at the University of California Hospital. Dietary studies have indicated in most instances borderline or frankly deficient diets with respect to vitamin C particularly in the winter months. Capillary resistance tests have revealed in general low levels which have risen on institution of diets high in vitamin C. It is of interest that in many cases there has been a decided delay in return of this index toward normal indicating the necessity of time for repair of a vascular injury. We do not feel that a diminished capillary resistance is an entirely reliable index of latent scurvy and its reduction in rheumatic fever cannot be assigned to this factor alone. Excellent weight gains have been recorded. One mild recurrence occurred in a child who repeatedly informed us that she was unable to secure the foods advised. A number of the patients have passed through acute upper respiratory infections without reactivation of the rheumatic process. The patients in our series have not yet been subjected to detailed analysis, and we do not consider the group large enough or the period of observation long enough for conclusion. To date, however, we feel that the study has been encouraging. A recent observation of Faulkner<sup>12</sup> is of considerable interest. He found that rheumatic children showing evidence of active disease almost uniformly gave a reticulated red blood cell response of from 3 to 5 per cent following administration of large doses of vitamin C. To me this suggests a deficiency.

#### DISCUSSION

I wish to make it entirely clear that the factor of infection in rheumatic fever is in no sense minimized. It appears equally essential in the rheumatic fever-like experimental disease. What the precise infecting agent is, if there is a specific factor in rheumatic fever, is not known. It may be one or many forms of the streptococcus, an unknown bacterium, or a virus. Much evidence points to the importance of the common pathogenic hemolytic streptococci. The mode of action is equally uncertain. It may operate through toxin production, through minimal localization or through an allergic mechanism involving antigen antibody reactions. Epidemiologic data, particularly the social distribution, strongly suggests a conditioning environmental influence. The experimental data implicate vitamin C deficiency.

*Essential Pathology of Rheumatic Fever*—Rheumatic fever is a disease fundamentally characterized by injury to connective tissues. Klinge<sup>6</sup> particularly has drawn attention to widespread connective tissue injury. A change which he has called "fibrinoid degeneration" he considers to be the basic lesion of the disease. It seems to me that substances included in this descriptive term "fibrinoid degeneration" are one of the following: (1) Swollen degenerated or necrotic collagen, (2) hyalinized fibrin, or (3) a combination of the two, that is, fibrin soaked collagen. Collagen degeneration or necrosis is seen in the valvulitis, the arteritis and at the center of the Aschoff body. Hyalinized fibrin may be present in any rheumatic lesion. It is usually most prominent in the subcutaneous nodules.

*Theory of Mechanism*—A consideration of the fundamental pathology of rheumatic fever and the experimental studies suggest a possible mechanism, operating in the development of the lesions. Vitamin C has been shown by the studies of Hojer<sup>12</sup> and Wolbach and Howe,<sup>14</sup> to be essential for the normal metabolism of connective tissue. In the presence of deficiency preexisting connective tissue substance undergoes degenerative changes and repair and replacement is either in abeyance or accomplished by an imperfect collagen. As the tensile strength of the finer vascular bed is dependent to a large extent upon the intercellular substances, collagen and reticulum the deficiency would impair their strength and render them more permeable. If such tissues were further insulted by a factor of infection it would not seem unlikely that they would suffer to a degree that would not occur in normal tissues. Such a concept would explain the acute necrosis or subacute degeneration of collagen that is a fundamental lesion of rheumatic fever. The increased permeability of small vessels would foster this injury of collagen. If the insult of the infecting factor upon the small vessels were great blood might escape into the perivascular connective tissues. This would explain the hemorrhagic manifestations commonly encountered in the disease. A lesser injury might allow only the escape of plasma from which fibrin would be deposited. The degenerated collagen, the fibrin or fibrin soaked collagen would act as a stimulus to repair. The reparative mechanism in the presence of a deficiency and a continued action of the injurious factor of infection would be imperfect and manifest itself by pathologic hyperplasia of surrounding connective tissue cells as seen in the Aschoff reaction.

*Existence of Latent Scurvy*—Considerable evidence has accumulated indicating that latent scurvy is probably more frequently present in children than is appreciated. Gothlin<sup>15</sup> using reduced capillary strength as an index of latent scurvy found evidence of vitamin C undernutrition in 18 per cent of school children in the province of Uppland (Sweden) during the months of April and May. Dalldorf,<sup>16</sup> working in New York, similarly using capillary resistance tests as a criterion considers that mild degrees of vitamin C deficiency may constitute a problem of considerable public health importance. If dietary histories can be relied upon, many of the rheumatic children in our series were on suboptimal diets, some frankly deficient in vitamin C. A recent survey of food purchases of families on relief in a California city<sup>17</sup> revealed inadequate purchase of vitamin C containing foods in a large number

of cases even though the survey was conducted in a season when these foods were plentiful and available at low prices.

*Metabolism of Vitamin C.*—The ability of the body to store vitamin C is limited. Even without a dietary source of the vitamin, there is a continued excretion of small amounts in the urine. Factors which may deplete the stores of vitamin C are of great interest and probably of great importance. Our own experience from experimental observations is that certain infections may act in this way. Harde and Benjamin<sup>18</sup> have presented brief experimental evidence that infection may deplete the organic stores of vitamin C. Van Eekelen and Kooy<sup>19</sup> have shown that fatigue may operate in a similar fashion. If acute infection depletes the organic store of vitamin C, it is of great importance to the concept presented. Under such circumstances, a mild degree of deficiency might by infection be rendered significantly severe in a relatively short period of time. Such a mechanism might afford a partial explanation of the latent phase noted by many observers between the acute upper respiratory infection and the clinical onset of rheumatic fever. Practically nothing is known of factors which may hinder or prevent absorption or utilization of vitamin C. In light of our limited knowledge of the metabolism of the vitamin interpretations based on studies of urinary excretion must be made with great caution. Harris and Ray<sup>20</sup> have shown that the normally nourished individual promptly excretes relatively large amounts of vitamin C following a large dose. On the other hand, deficient individuals show a lag in excretion. These observations are of considerable interest but conclusions based upon this type of study must be guarded. This method is obviously only a measure of immediate "saturation" level of the individual and gives no indication of a preexisting deficiency. Further, in the presence of a complicating factor as an infection it is probably not safe to assume that high excretion following test doses of vitamin C is an index of saturation. If infection may deplete the store of vitamin C, the ability of the body to utilize the vitamin may be disturbed.

What may we expect of vitamin C therapy in rheumatic fever? If vitamin C deficiency prepares the soil for an infection to produce the rheumatic injury, it is apparent that the deficiency then is only a contributory influence. If we consider the widespread and frequently severe character of this injury it will be appreciated that repair will require time. Further, it would seem that the reparative mechanism would probably be more or less ineffective during the active phase of injury. In severe cases it would appear that much of the finer vascular bed and connective tissues would need to be rebuilt and that these tissues would be susceptible to injury at any time until the restitution were complete. It will be seen then that we are dealing with something more complex than a simple deficiency. Time for repair would probably vary depending upon the extent and intensity of the injury. Following severe cases, a period of months in which the tissues were gradually supplied with vitamin C and freed of injury, it might be hoped for reestablishment of normal tissue anatomy. In certain pathologies, as valvular deformities, work on the restoration of the normal anatomy. The most that could be achieved would be of tissues which they could resist

the injurious influence of infection. The ability of vitamin C to do this can only be evaluated on the basis of prolonged clinical observation.

Two approaches to the solution of this problem suggest themselves. First a preventive type study to determine if optimal vitamin C nutrition over an adequate period of time will prevent susceptible groups from getting rheumatic fever. Likewise, a study of rheumatic children could be instituted to determine if a high vitamin C intake would materially reduce the tendency to recurrence. Particularly in this case should the element of time be carefully considered and judgment of effectiveness withheld until the period of observation had been long. The experimental and other data which have been outlined clearly, indicate the importance of such a clinical study.

#### SUMMARY

The concept that rheumatic fever may be due to the combined influence of vitamin C deficiency and infection rests upon a broad experimental basis. In guinea pigs, under this dual influence, lesions comparable to those of rheumatic fever may develop in the heart and joints. The not infrequent occurrence of subcutaneous nodules appears to complete the pathologic similarity. It is of interest that in the experimental work no sharp line can be drawn between a disease picture resembling rheumatic fever and one characterized by a chronic joint disability with pathologic similarities to atrophic (rheumatoid) arthritis. There is much evidence indicating a relationship between the two diseases as seen in man.

Epidemiologic data seem to support the thesis advanced. Particular significance is attached to the abnormally high incidence of rheumatic fever in the poor.

Clinical studies in progress have afforded encouraging data but are too few, and the period of observation too short to afford a basis for judgment.

Rheumatic fever is a disease fundamentally characterized by widespread injury to collagen. Based upon the experimental studies and pathologic anatomy of rheumatic fever, a theory of mechanism of the development of the rheumatic lesion is advanced.

Our knowledge of the metabolism of vitamin C is in the process of development. Capacity to store the vitamin is limited. Fatigue and certain infections may deplete the organic reserve. Factors which might inhibit absorption or utilization of vitamin C are not known. Urinary excretion studies may serve as an index of the immediate "saturation" level but are not a gauge of preexisting deficiency. The possible influence of infection in modifying the storage and excretion or utilization of vitamin C is not known. For these reasons data based upon urinary excretion must be interpreted with care.

The question of what we may reasonably expect from vitamin C therapy in rheumatic fever is considered in the light of the fundamental pathology of the disease. Even though vitamin C deficiency may contribute to the development of the rheumatic lesion, it is only one factor. Some influence of infection also operates. In view of this, and the frequently severe injury

accompanying the disease, judgment of the preventive or therapeutic effectiveness of vitamin C administration in rheumatic fever can be based only on prolonged clinical study.

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## DISCUSSION

DR. HOMER F. SWIFT, NEW YORK, N. Y.—Dr. Rinehart's report two years ago induced us to repeat it. The concept of synergic action of infection and some other factor is a useful hypothesis. Dr. Schultz, in our laboratory, has been able to confirm practically all of Dr. Rinehart's statements. One must ask, however, whether the experimentally induced lesions are truly rheumatic, or merely exaggerated scorbutic tissue changes. Thinking that the relationship between vitamin C deficiency and rheumatic fever would probably be revealed better in the clinic, Drs. Schultz and Sendroy have studied a group of patients in the Rockefeller Institute Hospital. Their results may be summarized as follows: No significant difference was found in the metabolism of vitamin C in rheumatic and non-rheumatic subjects. Fifty-seven previously rheumatic children were divided into two

groups, one received capsules containing 100 mg of vitamin C daily in the form of Redoxon, the other a placebo. The relative incidence of mild rheumatic relapses among the patients in these two groups was the same, and three of the first group developed severe rheumatic relapses. Fifteen patients with acute active rheumatic symptoms were given 250 mg of vitamin C daily without any observably favorable influence.

Although many of the rheumatic patients gave evidence of subnormal concentration of vitamin C at the time of admission to the hospital, others apparently had normal amounts in their bodies. Our clinical studies failed to prove the hypothesis that there was a causal connection between vitamin C deficiency and the symptoms of rheumatic fever. In view of the recently announced discovery of an antinfectious vitamin in fruit juices, it is possible that studies with pure synthetic vitamin C might have given different results from those obtained by the use of citrons fruit juices.

DR. M. J. SHAPIRO, MINNEAPOLIS, MINN.—During the past year I have carried on studies on possible vitamin C deficiency in rheumatic children. The criteria for a diagnosis of latent or subclinical scurvy are not at all clear. It is impossible to make such a diagnosis on patients on clinical findings alone. My work was carried out in the following manner. The habitual diets of 70 children who were known to have rheumatic fever, and of their families were investigated by a trained dietitian. Thirty-nine of these patients were on a diet which was sufficient in vitamin C content while 31 were on a diet which was definitely insufficient. The capillary resistance of the 70 patients was studied. A full mouth x-ray was taken, as well as an x-ray of a knee and a wrist. The capillary resistance test is not standardized and difficult to interpret. The various writers on this subject have used different methods for determining capillary resistance. Some have used simply the tourniquet for three minutes, others have used the blood pressure manometer at 50 mm for fifteen minutes and still others 80 mm for three minutes while Dalldorf has used his so-called capillary resistometer which is based on occluding pressure and takes only one minute to perform. These various methods were tried and an attempt made to correlate them. We decided to use Dalldorf's method as it is simplest and reliable. It was noted that of the 39 children who had a sufficient diet 24 or 61 per cent had a positive capillary resistance test while 15 or 38 per cent were negative and of the 31 children who were on an insufficient diet 19 or 61 per cent had a positive capillary test while 12 or 39 per cent had a negative test. It is well to state that there is no correlation whatsoever, between the capillary resistance test and the deficiency of vitamin C in the diet. The factor of toxicity in these rheumatic children as a cause of the capillary permeability must be considered. One is unable to say whether or not the decrease in capillary resistance is due to toxicity or vitamin C deficiency. My preliminary report would indicate that capillary resistance and vitamin C deficiency are not definitely related.

We based our findings in the teeth on the work done by Westin and Hoyer of Sweden. I was unable to show any relationship between vitamin C deficiency as indicated in the study of the diet and the findings in the teeth. Of 39 children on a sufficient diet 15 or 39 per cent were positive while 23 or 60 per cent were negative for dental changes. Of 31 children on an insufficient diet 12 or 40 per cent had positive findings in the teeth while 18 or 60 per cent had negative findings. In no instances did roentgenograms of the joints show definite evidence of latent scurvy. One meets with considerable difficulty as the roentgen findings in subclinical scurvy are not clear. We did find some unusual changes in the bones in a number of these children but consultation with Dr. Leo Rigler, the head of the x-ray department of our university, failed to establish a diagnosis of scurvy. Some of these films were sent to Dr. Bromer of Philadelphia and he also refused to commit himself on these findings. My preliminary investigations thus suggest that there is no relationship between vitamin C deficiency and juvenile rheumatism.

DR. WILLIAM J. KERR, SAN FRANCISCO, CALIF.—The work of Dr. Rinehart and the original work with Drs. Mettler and Connor have been of great interest. The histologic findings resemble closely those seen in rheumatic fever, and perhaps those in the proliferative type of arthritis. It is not so easy to carry results over to the clinic and say that

deficiency of vitamin C is a factor in the production of rheumatism or that we can immediately control the process by substituting vitamin C.

The work which Dr. Swift and his associates reported last month seems to be quite negative so far as the clinical application of the principle is concerned. But we should be sure that we have studied an adequate number of cases over a period of years before we can be quite certain of the results. We must also be sure that whatever substances we are administering actually enter the body. If these substances are given by mouth, conditions for their proper absorption must be satisfied. If there is any uncertainty about absorption we should give them parenterally. As I recall Dr. Swift's report, vitamin C was given intravenously in some instances. If there are other substances in fruit juices, which may be important factors, we should take them into account in treatment.

Dr. Rinehart and his clinical colleagues expect to continue this study for some time, not only on patients with rheumatic fever but also on those with atrophic arthritis.

DR. JOHN R. MOTE, BOSTON, MASS.—At the House of the Good Samaritan we repeated with minor variations the work of Dr. Rinehart on guinea pigs. Briefly it may be said that the results were essentially the same as those of Dr. Rinehart. The animals which had scurvy with an added streptococcus infection showed the most marked lesions in most instances. In the chronic scurvy controls we found the same type of lesion but of a less severe type. In the group of acute severe scurvy controls we found lesions of the type of the scurvy plus infection, which in most instances were not so severe, but in some just as widespread and severe as in the experimental group.

We have not been convinced that the lesions are either identical or even closely similar to those of rheumatic fever.

DR. J. A. KEY, ST. LOUIS, MO.—About fifteen years ago Dr. Howe produced arthritic changes in the joints of animals by diet. Dr. Wolbach found the changes represented atypical scurvy. I have studied guinea pigs on a mild scorbutic diet. Some of them developed changes in joints; first a shedding of the synovial lining cells. I wonder about Dr. Rinehart's controls and whether a similar series of animals kept on the same diet would develop the joint changes without injections of streptococci. In my own experience when streptococci are injected into animals they either develop a purulent arthritis or the joints are unaffected. I have not produced therewith a condition resembling chronic atrophic arthritis, and I do not believe that any guinea pig, rabbit, or other laboratory animal ever develops rheumatic fever or atrophic arthritis. So where are we going to get animal streptococci to produce these diseases in animals?

DR. WALTER BAUER, BOSTON, MASS.—If a lack of vitamin C plays a rôle in the causation of rheumatic fever, why is the incidence of rheumatic fever no higher in infants suffering from scurvy?

I am very doubtful that vitamin C deficiency plays any important rôle in the production of atrophic arthritis for the following reasons: For the past five years each patient discharged from our hospital with the diagnosis of atrophic arthritis has been instructed to adhere to a high vitamin diet so far as his pocketbook will allow. The dietary calls for one eight-ounce glass of orange juice each day and one eight-ounce glass of tomato juice each day. Many of these patients have adhered to this diet for from three to five years, yet the results in this group are no different than in the group who did not adhere to the diet. If what Dr. Rinehart says is true, one would expect to see a difference in the therapeutic results in these two groups.

DR. HOMER F. SWIFT, NEW YORK, N. Y.—Investigators studying artificial inoculation of laboratory animals with streptococci should understand the advantages to be derived from a knowledge of the serologic groups to which the particular strains they are using belong. Members of groups which are spontaneously pathogenic for man are generally not spontaneously pathogenic for animals, and groups pathogenic for animals have little disease inducing capacity for man. A common source of error is that we use so-called "human strains" to inoculate laboratory animals and expect these animals to have the same reactions as man. This was doubtless done in the experiments mentioned by Dr. Key;

but in Dr Rinehart's work a group of strains was employed, a strain that is normally pathogenic for guinea pigs, and one that satisfactorily induces chronic lesions in these animals. This is a good example of using the proper experimental agents in conducting experimental studies.

DR ERNEST E IRONS (Chicago, Ill.)—Some years ago we were studying chronic arthritis in hogs from which two organisms were isolated, a streptococcus and a short bacillus described in veterinary medicine. We inoculated a litter of little pigs with these organisms and had no trouble in producing chronic arthritis with persistent large articular deformities. In other laboratory animals no arthritis resulted from inoculations. As Dr Swift stated, to get characteristic lesions in animals the organism used must be suited to the animal inoculated. To me this has always seemed to be one of the weak spots in animal experiments in respect to human disease.

DR RINEHART (closing)—The tables of Dr Wollart referred to by Dr Key have shown that vitamin C is essential for the normal growth of connective tissue and the formation of normal intercellular substances. His experiment was designed to demonstrate this fact, and dealt essentially with the role of vitamin C in the reparative process following administration of the vitamin. His experiments do not differ materially from those which I have reported. We have controlled all factors except the effect of deficiency alone, the deficiency being induced with infection in the presence of adequate nutrition. The guinea pig, which is the only animal in which vitamin C deficiency alone develops a chronic arthritis, is the only animal in which arthritis develops. It is probable that Dr Howe's animal experiments, in which he has shown the pathologic picture is truly that of rheumatism, are the only experiments except of the disease. The rôle of infection is not clear, but it is probably related to the deficiency, the arthritis may be ascribed to the deficiency alone. This is not uniformly so, depending on the type of infection used. On the other hand, the various infections used in the experiments of Dr Swift and Dr Mote, which did not produce arthritis, are not the same as those used in the experiments of Dr Swift and Dr Mote.

I am glad that Dr Swift and Dr Mote have shown that the organisms derived from animal sources in animal experiments are not the same as those derived from spontaneous infections. Dr Mote's studies have not been made with organisms from other sources, but his studies are in addition to the vitamin C deficiency experiments of Dr Swift. The picture resembling rheumatic fever in the experiments of Dr Swift and Dr Mote, for the species, we have found this to be the case.

Dr Swift notes that our experiments in the laboratory. On the basis of studies of the metabolism of vitamin C in therapeutic studies, he concludes that the deficiency of vitamin C is an important determining factor in the etiology of rheumatism. He stated that our knowledge of the metabolism of vitamin C is not yet complete, but that by a factor of infection. Under such conditions, the deficiency of vitamin C may not denote saturation. It may be in excess of the body's ability to utilize the vitamin. Does the rheumatic process, as we call it, require the utilization of vitamin C? In pernicious anemia, certain infections, and the utilization of liver extract, necessitating great increases for an effective cure. Even though we could be assured that excretion of vitamin C impeded saturation of tissue, this would give us no index of a past episode of deficiency as a basic contributory factor. With respect to a lack of immediately measurable improvement following the administration of large amounts of vitamin C, in cases of rheumatic fever, it would seem unlikely that saturation of the body with the vitamin would quickly restore a tissue change that has been required over a period of years or has resulted from repeated insult. A widespread and frequently severe connective tissue and vascular injury is found in rheumatic fever. The exact mechanism of this injury is unknown. Even though vitamin C deficiency may be a factor in the initial mechanism, another influence, probably infection, also operates. If this injury can be



repaired by vitamin C, time would appear essential. I wish strongly to emphasize the importance of this in the evaluation of clinical protective or therapeutic studies. In severe cases it would appear that much of the finer vascular bed and its connective tissue support would need to be reconstructed and this repair could probably start effectively only after cessation of the injury.

Dr. Shapiro has questioned the value or meaning of capillary resistance tests. We are aware of the limitations of this test as an index of latent scurvy. However, repeated observations over long periods of time indicate its significance. The capillary strength in general is reduced in rheumatic fever. Although the levels of capillary strength rose on administration of generous amounts of vitamin C, in many cases this return toward normal levels required several months, indicating a delayed restitution of tissue injury.

Dr. Bauer notes that his patients with arthritis have, on discharge, been instructed to take generous amounts of vitamin C and does not feel that improvement in this group is significantly different than other groups. Most diets recommended in arthritis contain liberal sources of vitamin C. We only suggest that subacute or chronic vitamin C deficiency may be one mechanism or a contributory factor in the development of atrophic arthritis. In some cases we may have to look for abnormalities in assimilation or utilization of vitamin C. The profound circulatory and other anatomic changes which characterize the disease in its subacute or chronic stage, even though vitamin C lack had contributed to their development, would be slow to respond to the administration of the vitamin. A prolonged clinical study of this phase of the problem is needed. Dr. Bauer also asks why the incidence of rheumatic fever is not high in scurvy. I do not know of any analysis of scorbutic cases from this standpoint. Clearly recognizable scurvy in this country is almost limited to infancy. It is not improbable that a childhood deficiency, perhaps more chronic and less intense, but just as real, would not be recognized by our usual standards.

In conclusion, I wish to reaffirm the broad experimental basis for the concept presented and to stress the necessity for a prolonged clinical study as the basis for final judgment.

## INFLUENCE OF THE TONSILS ON RHEUMATIC INFECTION IN CHILDREN

ALBERT D. KAISER, M.D., ROCHESTER, N. Y.

**R**HEUMATIC disease with its various manifestations has been clinically associated with tonsil infections for a considerable period of time. Extensive literature on this subject has appeared which has not been in entire agreement. Tonsillectomy in selected children seemed to be followed by the subsidence of rheumatic manifestations and consequently the method of treatment was heralded as most successful. Such treatment was generally instituted until its practice became almost universal. If we consider the results seen in selected children were not always apparent when the operation was more commonly performed, so that the pendulum has swung to the other extreme questioning whether tonsillectomy ever has been a really effective treatment in the prevention of rheumatic disease.

A review of the literature on the influence of the tonsils on rheumatic infection leaves one in doubt as to the effect of the tonsils in the causation of rheumatic disease. There seems to be agreement that tonsillar infection in the tonsils frequently precedes the development of rheumatic disease. On the other hand, many children subject to tonsillar infection do not develop evidences of rheumatic disease.

Assuming that infection in the tonsils is a necessary condition in children who develop rheumatic disease, three questions arise: First, what is the incidence of rheumatic infection in children whose tonsils have been removed before rheumatic infection sets in? Second, does removal of the tonsils influence the course of rheumatic infection when the disease has been established? In other words, are there fewer recurrences of the disease? Third, what is the influence on the absence of the tonsils influence the outcome of this disease? Fourth, is the death rate increased or diminished by the treatment of the tonsils? If a satisfactory answer could be found for these three questions it would be possible to state something definite about the influence that the tonsils exert on this disease.

In order to answer the first question on the incidence of rheumatic infection in children whose tonsils have been removed and in those whose tonsils have not been removed one must have information on a large group of children over a considerable period of time. A number of years ago a survey was undertaken in Rochester to obtain information on the general incidence of rheumatic manifestations in all of the school children. This information was obtained from the parents of the children. The parents of 48,000 children were inter-

\*Read at the Second Annual Meeting of The American Association for the Study and Control of Rheumatic Diseases and the Fourth Conference on Rheumatic Diseases, June 10, 1935, Atlantic City, N. J.

viewed. Of this number, 20,000 children had been tonsillectomized and 28,000 had not had their tonsils removed. The information obtained in this survey stated the number of children who at some time during their life had clinical evidence of rheumatic infection. It was impossible, however, to determine whether the first rheumatic manifestation preceded the tonsillectomy, if done, or whether it occurred after the tonsils were removed. It did nevertheless give a general idea of the incidence of the various rheumatic manifestations in children.

Based on these data as obtained from the parents nearly all of the rheumatic manifestations occurred less commonly among tonsillectomized children. Rheumatic fever which is usually a severe type of rheumatic infection was reported with considerably less frequency in the tonsillectomized children. Among the children with their tonsils out there were 37 per cent fewer cases of rheumatic fever. Muscular rheumatism, termed growing pains, was reported only slightly

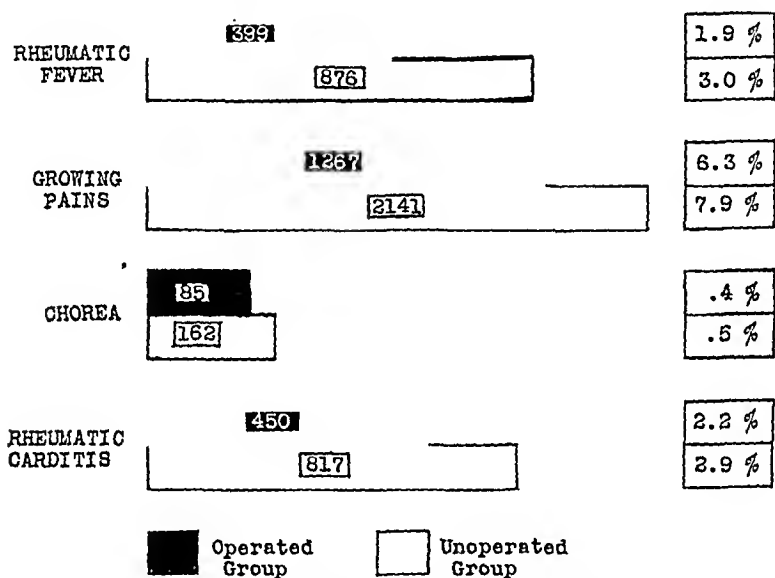


Fig. 1.—Chart showing comparative incidence of rheumatic manifestations in children on whom tonsillectomy has and has not been performed (based on the history of 48,000 school children of whom 20,000 had undergone tonsillectomy and 28,000 had not).

less often in tonsillectomized children. Chorea was noted with equal frequency in the two groups while rheumatic carditis was somewhat less common among the children with their tonsils out. In this survey many children among the tonsillectomized group reported some rheumatic manifestation. In some of them the rheumatic infection began before the tonsils were removed. If it had been known whether the tonsillectomy preceded the rheumatic infection or followed it, a more favorable comparison for the operated group might be noted. This statistical information based on the parents' history of the child leaves some uncertainty in the value of the data. It does, however, clearly indicate that rheumatic disease occurs in children whose tonsils have been removed, and it also seems highly probable, as noted in Fig. 1, that initial attacks of rheumatic infection are somewhat less likely to develop in children whose tonsils have been removed.

More accurate information dealing with the initial infection and the presence or absence of tonsils was obtained from an analysis of 439 children with acute rheumatic infection who were observed over a five year period from January, 1925, to January, 1930. In addition with the number of children whose tonsils have been removed at various ages it was possible to compute the expected incidence of rheumatic disease among tonsillectomized children in the community.

TABLE I  
COMPARISON OF THE ACTUAL WITH THE EXPECTED PERCENTAGE OF TONSILLECTOMIZED CHILDREN  
AMONG 439 CASES OF ACUTE RHEUMATISM

AGE OF CHILDREN	TOTAL NO CASES	EXPOSED NUMBER & PHARYNGITIS CASES WITH TONSILLOPHARYNGITIS	ACUTE LUMPY PHARYNGITIS CASES WITH TONSILLOPHARYNGITIS	NUMBER OF PHARYNGITIS CASES THAT DEVELOPED SECONDARY TONSILLOPHARYNGITIS	PER CENT OF ROCHESTER CHILDREN WITH TONSILLOPHARYNGITIS
Under 5 years	38	8	2	6	10
5 10 yr	126	120	21	101	33
10 15 yr	239	113	42	151	50
16 17 yr	36	19	11	20	55
Total	439	159	76	177	

For all ages, as noted in Table I the incidence of children who developed rheumatism after the tonsils were removed was considerably less than the expected rate. The difference between the actual and the actually expected upon and the expected percentage is 14 per cent. Utilizing an accepted formula for determining the difference between the actual and the expected, that the difference, 14 per cent is significant. At least one third more children figured on a percentage basis as having developed rheumatism when the tonsils were in than those who had them removed. The full value of removing the tonsils in the case of children who have rheumatism can not be ascertained from the study of this material. This has been done by various authors. If tonsillectomy is done in the homes, schools and cities over the same period of time, it will show fewer cases of rheumatism than a like group untreated. This is the basis of the belief that early enucleation of the tonsils has protective value against this infection. Such has been the case in large groups of children in Rochester.

Additional information was obtained on the subject of the tonsils in the prevention of the various rheumatic manifestations from a study of 4400 children. Composed of two equal groups tonsillectomized and nontonsillectomized, the various rheumatic manifestations could be compared inasmuch as these children were observed for a period of ten years.

As noted in Fig 2 chorea was known to have existed in 7 of the 2,200 children who were subsequently tonsillectomized. An equal number of children with chorea were found in the group of 2,200 children who were not later tonsillectomized. During the ten year period following tonsil removal, 26 children developed chorea, while among the 2,200 children not tonsillectomized 13 developed chorea. The seriousness of chorea can be judged by the occurrence of carditis associated with chorea. The incidence of carditis in the children who developed chorea

while the tonsils were in was 62 per cent. Among the children who developed chorea after the tonsils were removed, the incidence of earditis was 47 per cent. The inference is that chorea is not influenced favorably with removal of the tonsils, except in safeguarding slightly against the serious complication of rheumatic earditis.

Incidence before Operation  
4 - 7 years of age

Incidence after Operation  
14 - 17 years of age

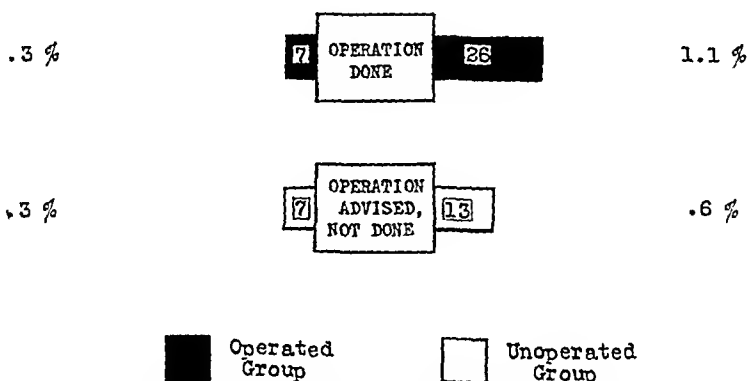


Fig. 2.—Incidence of chorea in 2,200 children before tonsillectomy and ten years after operation as compared with an equal number of controls.

Incidence before Operation  
4 - 7 years of age

Incidence after Operation  
14 - 17 years of age

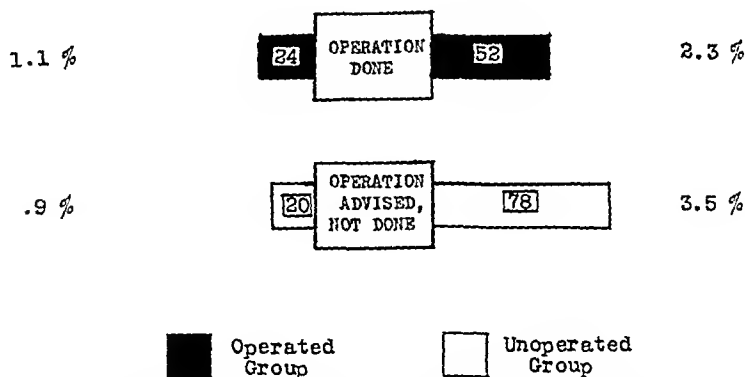


Fig. 3.—Incidence of rheumatic fever in 2,200 children before tonsillectomy and ten years after operation as compared with an equal number of controls.

The incidence of rheumatic fever was studied in the same two groups as shown in Fig. 3. Before removal of the tonsils 24 or 1.1 per cent of the 2,200 children had been afflicted with rheumatic fever, while among the control children, 20 or 0.9 per cent were similarly affected. During the ten-year period following tonsillectomy, 52 children or 2.3 per cent developed rheumatic fever, while 78 or 3.5 per cent of the control children were likewise infected. Realizing

that rheumatic fever occurs more commonly between five and ten years of age than before the fifth year. There is no reason to believe that in this series enucleation of the tonsils was responsible for a lower third fever cases of rheumatic fever than in a similar group whose tonsils and adenoids were not removed. As with choera, the occurrence of rheumatic fever was studied. In the children who developed rheumatic fever for the first time after tonsil removal 69 per cent developed rheumatic endocarditis. In this study it seems reasonable to conclude that removal of the tonsils offers some hope for escape from rheumatic fever. It occurred from 25 to 35 per cent in the non-tonsil-enucleated children. The serious complication of rheumatic endocarditis should develop in children with rheumatic fever whose tonsils are not removed, even when the tonsils are still present.

Though there is some doubt as to whether the frequency of growing pains

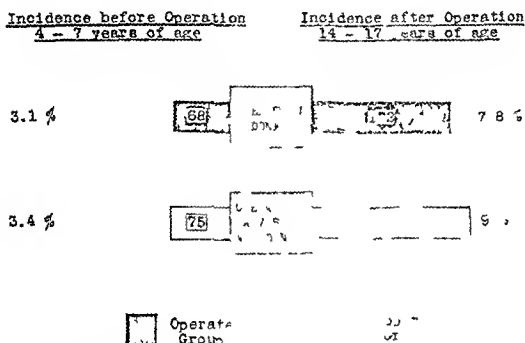


Fig. 4—Incidence of growing pains and ten years after operation.

1 tonsillectomy

or muscular pains to rheumatism are mild manifestations of rheumatism. With reference to the tonsils, the incidence of growing pains as shown in Fig. 4 interpreted with equal frequency in the operated and unoperated groups. During the school age period 172 tonsillectomized children were subject to this complaint while 200 of 9 years of age and 10 tonsillectomized children were having growing pains. The less frequent occurrence of this complaint is recognized in the infrequent complication of rheumatic endocarditis was detected in 16 per cent of the children subject to muscular pains in whom the tonsils had not been removed and in 18 per cent of the children whose growing pains manifested themselves for the first time after the tonsils were removed. The milder manifestations of rheumatism known as growing pains or muscular pains seem to be less influenced by the absence of tonsils than is rheumatic fever. It occurs somewhat less frequently in tonsillectomized children but endocarditis is as likely to follow this mild rheumatic infection after the tonsils are removed.

Rheumatic carditis was studied in the 4,400 children similarly to the other rheumatic manifestations. During the ten-year period in which these children were observed, it developed that 1.1 per cent of the children contracted rheumatic carditis following tonsillectomy, while among the children whose tonsils were not removed, 1.3 per cent contracted rheumatic carditis. The difference is not significant. If one judges the value of tonsil enucleation to children who have had an attack of rheumatic fever, there is little to commend the operation for the control of rheumatic carditis. If, however, one analyzes a large child population where the incidence of rheumatic fever and chorea is known, it is evident that slightly less carditis exists in tonsillectomized children. This lessened incidence is due to fewer cases of rheumatic fever among tonsillectomized children, and a somewhat diminished incidence of carditis following chorea in tonsillectomized children. These three separate studies justify the statement that the tonsils have

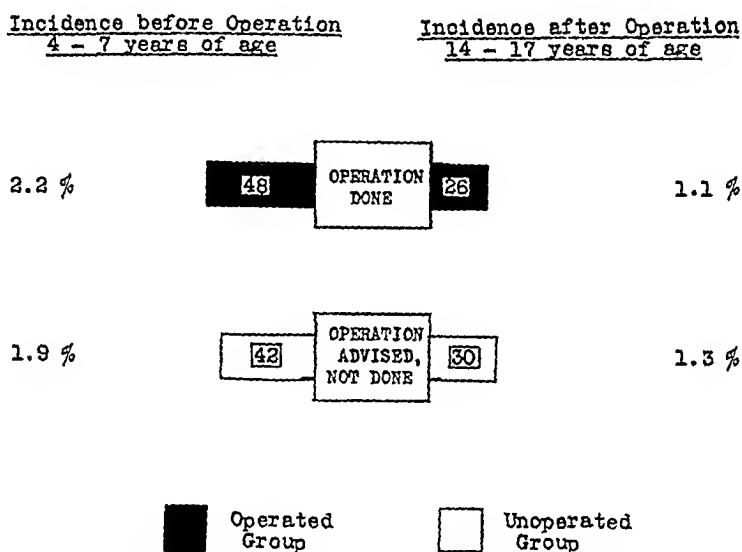


Fig. 5.—Incidence of rheumatic carditis in 2,200 children before tonsillectomy and ten years after operation as compared with an equal number of controls.

some influence on the incidence of rheumatic disease as recognized in children. They agree that rheumatic fever occurs about 30 per cent less often in children whose tonsils have been removed; that chorea occurs as often in tonsillectomized children as in those not so treated; that muscular rheumatism occurs slightly less often in the tonsillectomized children; that rheumatic carditis is found less often in the children whose tonsils have been removed.

In answer to the second query, Can recurrent attacks be influenced by tonsillectomy?—adequate data are available from several sources. Wilson, Lingg and Croxford prepared an excellent study of 413 rheumatic children in 1928 to determine the influence of the tonsils on recurrent attacks. In their survey, a careful correlation was made between the age of tonsillectomy and the age at which recurrent attacks occurred. It was brought out that recurrences occur in young children under nine years of age whether tonsils are removed or not while after ten years of age, recurrences are less common in both groups. Because of

their results they conclude that the routine removal of tonsils for the prevention of rheumatic heart disease in children is not justified by conclusive data. Poynton, on the other hand, believes that excision of the tonsils tends to stop severe recurrent throat infections, which on each occasion lay the rheumatic patient open to a recrudescence of the disease. In a study made by the author on 439 children who had acute rheumatism the benefits of tonsillectomy in the prevention of recurrent attacks were not apparent.

TABLE II

INCIDENCE OF RECURRENT ATTACKS OF ACUTE RHEUMATISM WITH REFERENCE TO REMOVAL OF THE TONSILS IN 11 CHILDREN

AGE OF CHILDREN AT FIRST ATTACK	NUMBER IN WHOM FIRST ATTACK DEVELOPED BEFORE TONSILLECTOMY	NUMBER WHO HAD RECURRENCE OF ATTACKS	NUMBER IN WHOM FIRST ATTACK DEVELOPED AFTER TONSILLECTOMY	NUMBER WHO HAD RECURRENCE OF ATTACKS
Under 5 yr.	36	11—31%	—	1—50%
From 5 to 10 yr.	104	47—45%	—	10—45%
From 10 to 15 yr.	151	1—	5—	10—21%
16 and 17 yr.	25	2—	11—	4—36%

Recurrent attacks of rheumatism in the tonsillectomized children as in the untreated ones. In the untreated group between the ages of ten and fifteen, when recurrent attacks are most common, the figures are high. The figures noted in Table II do not describe the frequency of recurrent attacks but in a general way, agree with the results obtained by other workers. Although the number of recurrences of such manifestations was less in the tonsillectomized and muscular pains was not lessened by removal of the tonsils, the author concluded that carditis associated with recurrent rheumatism in the tonsillectomized group was somewhat less severe in the tonsillectomized group.

A solution was sought for the question of the influence of the removal of tonsils in the rheumatic child influence the outcome of the infection. In a recent study made in Rochester the outcome in 597 rheumatic children was observed. In almost comparable groups the mortality was 13 per cent among the children whose tonsils were in during their rheumatic infection and 7 per cent among those whose tonsils were out at the time of the initial attack. This result suggests that the most serious type of rheumatic infection is more likely to occur in children whose tonsils are still present.

Though the removal of the tonsils tends to decrease the number of recurrences of rheumatic infections, there is a decidedly lower mortality rate among those

TABLE III

THE EFFECT OF THE TONSILS ON THE OUTCOME OF RHEUMATIC INFECTION IN 597 CHILDREN

TONSILS	NUMBER	DIED	OUT OF HOPES	NO OUTCOME
Remained in	156	13%	45%	42%
Out at initial attack	187	7%	44%	52%
Out after initial attack	254	4%	—	—



children whose tonsils have been removed. If other studies show similar results, there is a definite indication in every rheumatic child for the removal of the tonsils.

There is statistical evidence based on controlled studies to justify the statement that tonsillectomized children are somewhat less likely to develop rheumatic manifestations. The lessened incidence of rheumatic infection is noted in cases of rheumatic fever and rheumatic carditis. Muscular rheumatism and chorea are as likely to occur in children whose tonsils have been removed as in those whose tonsils are still present.

The incidence of recurrent attacks of rheumatic manifestations is not influenced by tonsillectomy. If rheumatic disease occurs in children whose tonsils have been removed prior to the initial attack the number of recurrences is likely to be as great as in children whose initial rheumatic infection developed while the tonsils were still in. Where tonsillectomy was performed after the initial rheumatic infection the number of recurrences were no less than in the rheumatic children whose tonsils were not removed.

The end-result in rheumatic disease in children did show the influence of the tonsils. The mortality in nearly 600 rheumatic children was nearly twice as high in the children whose tonsils were in at the time of the initial attack. The absence of the tonsils apparently safeguarded some of the rheumatic children against the serious cardiac complications that are usually responsible for death.

Statistical and clinical data justify the removal of the tonsils in practically every rheumatic child until other factors that influence this disease are better understood.

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#### DISCUSSION

DR. RUSSELL L. CECIL, NEW YORK, N. Y.—Assuming that Dr. Kaiser's statistics are correct, that a tonsillectomized child has a 35 per cent better chance against having rheumatic fever, the question arises: When, in this climate, should tonsillectomy be done? I know of no more vexing problem to mothers in New York City whose children have repeated respiratory infections without joint manifestations, but with some fever and possibly some complications in the ears. An argument generally occurs between the pediatrician, the internist, and the laryngologist. The laryngologist is disposed to advise tonsillectomy in children earlier than the pediatrician or internist. I saw a three-year-old girl just a few days ago who had repeated respiratory infections, a little fever with each attack, tonsils that look infected, and involvement of the cervical glands. The question came up whether the tonsils should be removed at once, before any serious damage was done, or whether we should wait until she was five years old. In some respects it seemed best to wait, but I think a child who is having trouble with tonsils should have them removed before serious damage to other organs is done. In children with no more than the average number of respiratory infections, it is well to postpone tonsillectomy until they are five or six. I would like to hear from Dr. Kaiser on this important point.

DR. M. J. SHAPIRO, MINNEAPOLIS, MINN.—If Dr. Kaiser has concluded that tonsillectomy should be done in 100 per cent of children who have had rheumatic fever, I wish to take issue with him. All of us have seen serious even fatal flare-ups in quiescent juvenile

rheumatic disease immediately after a tonsillectomy. A definite diagnosis of rheumatic infection in children is often difficult to establish. If all children who complain of leg pains are subjected to tonsillectomy, we will only add a good number of useless tonsillectomies to the already large number of these operations being done constantly on insufficient ground. As a result of careful study of several hundred rheumatic children for a period of twelve years, I have concluded that tonsillectomy should be done no more often in them than in any other group.

DR KAISER (closing).—In answer to the question as to whether 100 per cent of rheumatic children should have their tonsils removed, I should say no. That is a rather small number after all, when one realizes that in any community almost 50 per cent of children are having their tonsils removed without reason. Private school health records show that 55 per cent have had tonsillectomies performed. The reason that so many children have tonsillectomies is that the pediatrician and the otolaryngologist advise operation for frequent colds. If one recommends tonsillectomy in all individuals in the so-called rheumatic group it will not run over 10 per cent. Tonsillectomy is most helpful to children with tonsillitis. It is in the rheumatic group that tonsillitis occurs. If we concentrated on that group we would have fewer disappointing results.

The most definite indication for tonsillectomy in children is a history of repeated attacks of tonsillitis. That is the one infection which most students of this problem believe leads to rheumatic fever. If we restrict our tonsillectomies to children who have tonsillitis, we will not have so many failures.

DISCUSSION ON PAPER BY DR. HUGH McCULLOCH,  
"INSTITUTIONAL PROVISIONS FOR THE CARE OF THE  
RHEUMATIC CHILD"\*

W. D. STROUD, PHILADELPHIA, PA.

AT THE Children's Heart Hospital in Philadelphia, we have taken care of about 600 children with rheumatic heart disease in the past fourteen years. Three cardiologists and three pediatricians have been associated in caring for these children. We have concluded that tonsillectomized rheumatic children have done better than those seen in other clinics who have not had their tonsils removed. One of our requirements for admission to the Children's Heart Hospital is that no child will be admitted who has not had his tonsils removed. In addition to tonsillitis being an indication for removal of tonsils in the rheumatic child or in any child, we feel that a tonsillectomy should be performed earlier in children belonging to rheumatic families. Such children should be considered eligible for tonsillectomy at about the age of three years. To the parents of all these 600 children we have urged tonsillectomy for the other children in the family.

We feel that institutional care is of real benefit to children with rheumatic heart disease. The two objections I have heard raised against such institutions are these: if we put into such an institution a child who is not really rheumatic, he is exposed to the etiologic factor of rheumatic fever, through close contact with the others suffering from the disease. In the past fourteen years, we have had two epidemics apparently produced by streptococcic sore throats in nurses. Of 50 children in the institution at the time, only 12 or 14 had exacerbations of their rheumatic heart disease.

It has been argued that children of rheumatic families may, through contact with the disease, gradually develop less sensitivity to its etiologic factor. Removal to an institution and away from the family environment, might interfere with this. Since in such institutions these children are surrounded by others apparently hypersensitive to the etiologic factor, I believe such a criticism is not valid.

Points in favor of such institutions are, first, the general physical improvement which these children develop. Our hospital is within the city limits, surrounded by Fairmount Park. In such an environment, the general physical status of the children has definitely improved. They receive prolonged rest and at the same time, since a teacher is in attendance, are able to keep up with their studies and return to their own class in school after leaving the hospital. Thus, they do not suffer the introspection and inferiority complex common in children with physical handicaps, who must be associated with children much younger than themselves. One of the main objects of the institution is the education of parents. On visiting days, the head nurse lectures to parents and distributes literature of the American Heart Association. The children and parents learn the principles of hygienic care. A visiting nurse and social service workers follow this type of education in the home.

Such institutions provide graduate and undergraduate medical education, and an opportunity for clinical investigation.

For three years we have been using intravenous vaccine. The children who have received the vaccine have done no better than the control group. During a mild epidemic of reactivations, apparently produced through exposure to a streptococcic sore throat, as many of the vaccinated children developed exacerbations, as those in the control or unvaccinated group. In an effort to reduce the hypersensitivity of children to the etiologic factor in rheumatic fever, Dr. Joseph Stokes at the Children's Hospital in Philadelphia has been using a serum from whole blood of persons past the age of thirty-five in non-rheumatic families. Some children seem to have done well following this procedure.

\*Read at the Second Annual Meeting of The American Association for the Study and Control of Rheumatic Diseases and the Fourth Conference on Rheumatic Diseases, June 10, 1935, Atlantic City, N. J.

## FEVER THERAPY IN CHOREA AND IN RHEUMATIC CARDITIS WITH AND WITHOUT CHOREA\*

LUCY PORTER SUTTON, M D, and KATHARINE G DODGE M D, NEW YORK, N Y

### FEVER INDUCED BY TYPHOID PARATYPHOID VACCINE

**D**URING the past five years fever therapy has been used on the Children's Medical Service at Bellevue Hospital as the treatment of chorea. We were led to investigate this form of therapy because of the striking disappearance of a severe attack of chorea in a boy who became intoxicated by luminal and developed a high fever. A review of the literature revealed that the forms of therapy reported to have had definite effect on the duration of chorea, i.e., milk injections, nuxvomol intoxication, and relapsing fever had one factor in common, the production of fever. It had also been frequently observed that intercurrent infections had a beneficial effect on chorea. It was therefore difficult to escape the conclusion that it was the fever itself rather than the instrument which produced the fever which was beneficial in chorea.

After trying intravenous injections of typhoid vaccine with varying results, we began using typhoid paratyphoid vaccine, with which we found we could produce fever almost at will. The reason that this vaccine was chosen as the means of producing fever was that it was cheap, easily available, reasonably safe and required no elaborate set up or special technique. A method of procedure was developed which was given in detail in a previous publication<sup>1</sup>. In brief, the patient receives New York City triple typhoid vaccine (containing 1,000 million *B. Typhosus*, and 750 million each of Para A and B per c.c.) intravenously, beginning with a dose of 0.05 or 0.1 c.c., subsequent daily dosage being determined by the reaction of the patient to the previous one. The aim is to obtain a temperature of 104° to 106°. If necessary, a second dose on the same day is given. Treatment is continued daily, with occasional days of rest, until all signs of chorea have disappeared. In the mild and moderate cases this is seldom difficult to determine but in some children, particularly in those with severe chorea, where there has been marked hypotonia, it may be hard to decide just when the incoordination is due simply to weakness rather than to chorea. In these cases the child receives massage and occupational therapy. If the weakness decreases with increased activity, we feel safe in saying the attack is over. If the incoordination becomes worse, treatment is resumed. Figs 1 and 2 show the temperature charts in two types of cases. Type 1 was a mild chorea, easy to treat, needing only small doses of vaccine to obtain the desired temperatures and responding well to a short course of vaccine. Type 2 was a severe chorea difficult to treat requiring a long course of treatment and large doses of vaccine. In between these two

\*From the Children's Medical Service of Bellevue Hospital and the Department of Pediatrics, New York University.

Read at the Second Annual Meeting of The American Association for the Study and Control of Rheumatic Diseases and the Fourth Conference on Rheumatoid Diseases, June 11, 1937, Atlantic City, N. J.

types lies the majority of the cases. The average number of treatments in the first 150 cases treated by this method was 6.24. In the mild cases the average was 5.14 treatments; in the moderate 6.47 and the severe cases 8.88. The minimum number of treatments was 3, the maximum 18.

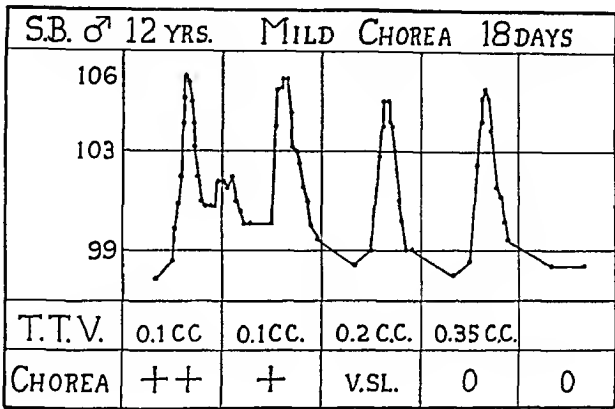


Fig. 1.

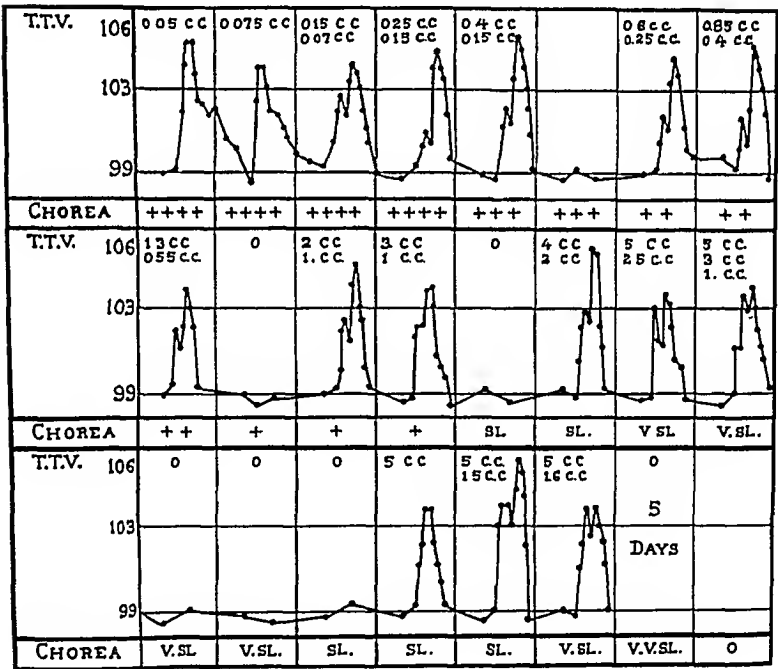


Fig. 2.

The results with fever therapy in the first 150 cases of chorea so treated have been previously reported.<sup>1</sup> For comparison we offered 150 cases treated on the same service before 1930 with various drugs, forms of physiotherapy and diet, plus rest in bed and isolation. Those who received fever therapy had definitely shorter attacks. The duration of chorea in the hospital in the group used for comparison, and the duration after beginning of fever treatment, are shown in Table I. This is also shown graphically in Fig. 3.

## FEVER INDUCED BY RADIANT ENERGY

The disadvantages of producing fever by the intravenous injections of triple typhoid vaccine are the occurrence of protein shock, which may be very severe following the first two or three injections, and the inability to control the fever or to maintain it at the desired high level for any appreciable length of time. Investigation of other methods of producing fever led us to the conclusion that the use of radiant energy as developed by Dr. Stafford Warren and his co-workers at the University of Rochester might prove superior to the intravenous vaccine method. We therefore had such an apparatus made, in which a patient's temperature may be raised to any desired level and maintained for as long a time as is necessary or the condition of the patient allows. Fig. 4 shows the type of temperature curve obtained by this means. Analysis of the first

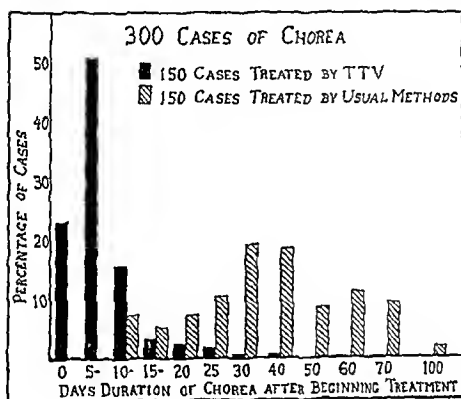


Fig. 3

TABLE I  
DURATION OF CHOREA

TYPE		NUMBER OF CASES	AVERAGE DURATION IN DAYS	RANGE IN DAYS
Mild	Comparative group	48	27.4	10-67
	Treated group	65	5.72	2-14
Moderate	Comparative group	68	44.0	15-120
	Treated group	57	8.56	3-22
Severe	Comparative group	33	62.4	24-180
	Treated group	25	15.8	5-47
Whole Series				
Comparative group		130	42.6	10-180
Treated group		150	8.5	2-47

sixteen cases treated by this method shows that the results in terms of the duration of the chorea after one or two treatments are comparable to those obtained with a course of foreign protein induced fever (Fig. 5). Certain advantages of this method may be mentioned: (1) The fever is controllable. (2) One or two

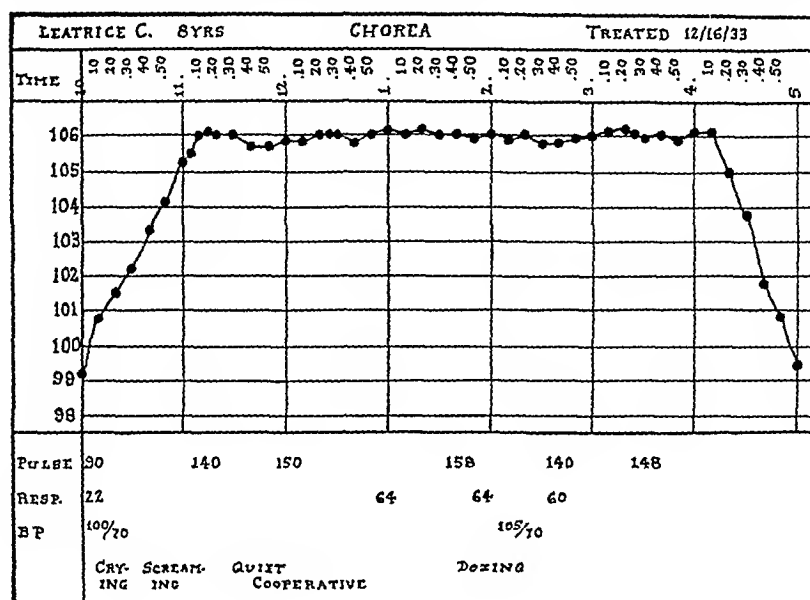


Fig. 4.

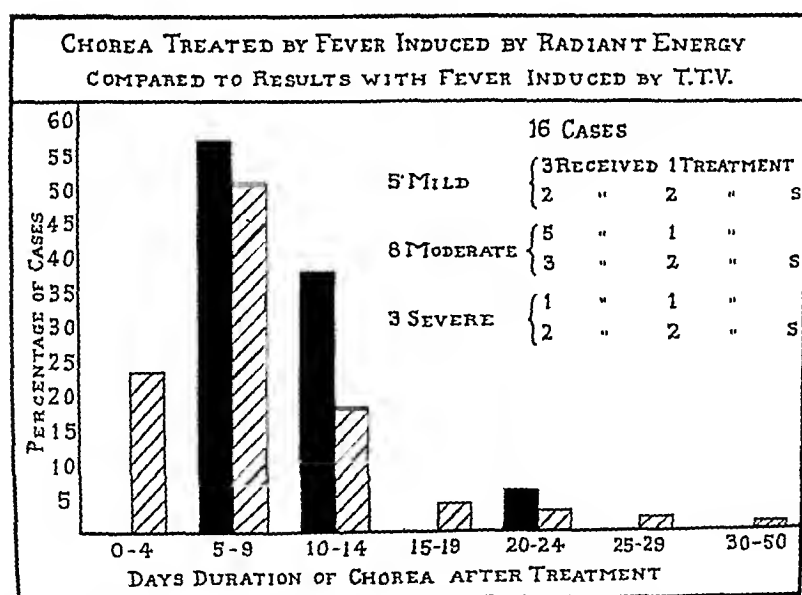


Fig. 5.—Solid black columns represent percentages of 16 patients treated by radiant energy. Cross-hatched columns represent percentages of 150 patients treated by intravenous triple typhoid vaccine.

treatments take the place of daily injections for a week or more of a foreign protein. (3) The patients appear distinctly less uncomfortable during treatment

with radiant energy than during treatment with foreign protein shock (4) Except for the day of treatment the burden on the nursing staff is much diminished. The treatment of a larger number of patients may modify our results and lead us to change our opinion.

#### THE EFFECT OF FEVER THERAPY ON RHEUMATIC CARDITIS WITH AND WITHOUT CHOREA<sup>3</sup>

Among the first 200 patients who received typhoid paratyphoid vaccine for chorea were 16 children with definite evidence of active rheumatic carditis at the time of treatment, and in addition 27 with clinically inactive heart disease. Among the latter group were 9 with mitral stenosis and insufficiency and 1 with both mitral insufficiency and stenosis and aortic insufficiency and stenosis. Our criteria for the diagnosis of active carditis were the presence of three or more of the following:

- 1 Fever
- 2 Tachycardia
- 3 Change in the quality of the heart sounds
- 4 Presystolic gallop sound at or just inside the apex
- 5 Development of new murmurs
- 6 Change in the quality of the murmurs present especially the presence of a high pitched shrill musical quality in the apical systolic murmur (some times described as "sea gull quality")
- 7 Change in rhythm (heart block or loss of sinus arrhythmia)
- 8 Electrocardiographic changes chiefly prolongation of the P R and QRS intervals
- 9 The presence of subcutaneous rheumatic nodules

The effect of fever therapy on the clinical signs of active carditis in 5 illustrative cases is shown in Table II. In the whole group of 16, 9 had lost their clinical signs of activity by the time the chorea was over and the treatment stopped, and the remaining 7 were clinically inactive within seven to ten days after the end of the treatment. We appreciate the fact that there are cases in which the signs of active carditis subside after a short time at rest in bed, but on the other hand the tendency to chronicity of the rheumatic infection is well known.

While we did not think that the disappearance of the signs of active carditis in the majority of cases by the end of treatment was necessarily the direct result of fever therapy, we believed that it was sufficiently suggestive to justify an investigation of the effects of fever on various forms of rheumatic heart disease without chorea. Up to the present time we have treated with fever produced by radiant energy seven children with subacute rheumatic carditis without chorea and one child with a very severe acute carditis accompanied by polyarthritis and chorea. These patients were given a fever of approximately 106° for from two to five hours at a time. Five of them received two treatments each. All of these children showed decreasing signs of active infection immediately or within a comparatively short time after treatment. Figs 6 and 7 show graphically the clinical course in the hospital of the first two patients treated by this means.



I. B. (Fig. 6) was seven and one-half years old when she was admitted in November, 1933. She had had polyarthrititis at five and six years, and is said to have had heart disease since the age of three years. The present illness began

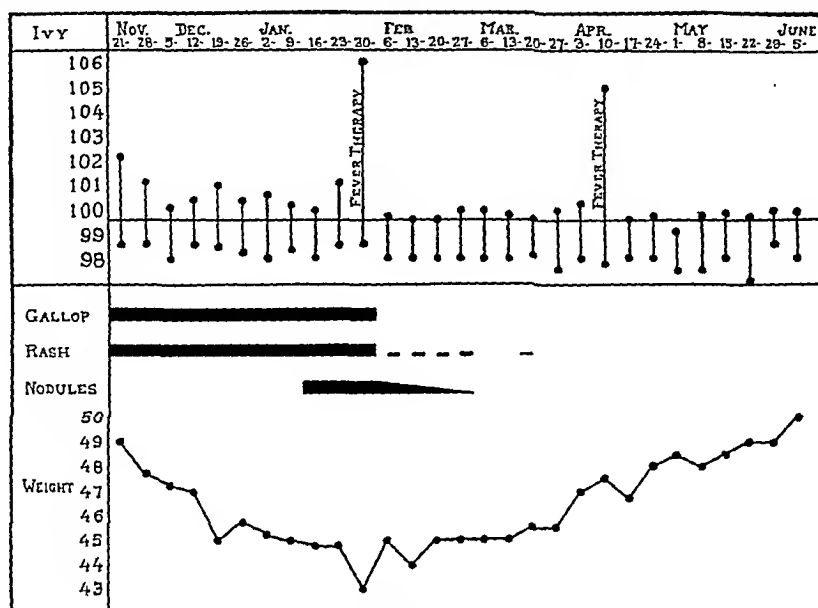


Fig. 6.

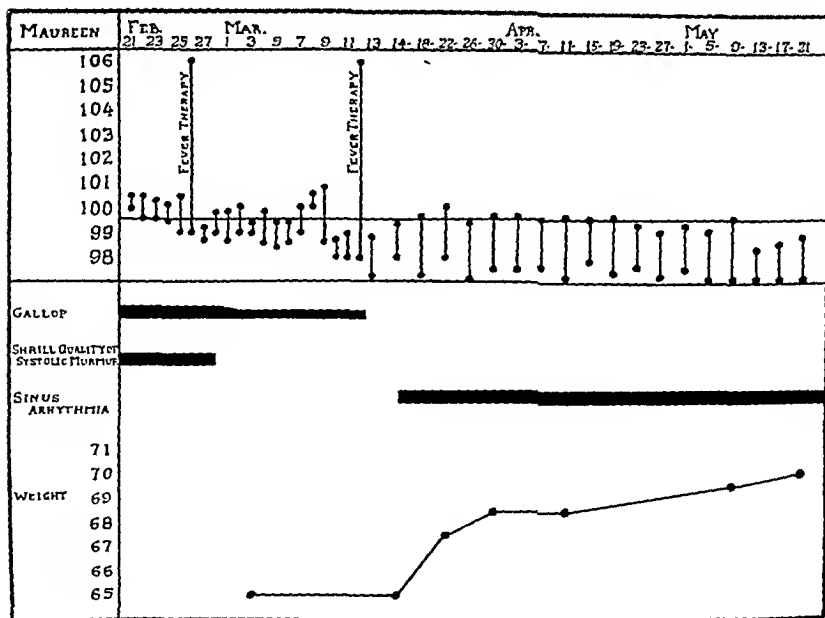


Fig. 7.

two weeks before admission with the development of an erythematous rash. Three days later she began to feel tired, to have migratory joint pains and afternoon fever. On admission she appeared acutely and chronically ill, her tempera-

ture was  $101^{\circ}$ , there was widespread erythema marginatum, her heart was enlarged, there was tachycardia, presystolic gallop, an accentuated P., a loud harsh systolic murmur at the apex transmitted to the axilla and base, and a low pitched diastolic murmur. The persistence of the signs of active infection during the first ten weeks of her stay in the hospital is shown in the chart. The striking subsidence of these signs following one fever treatment of four hours between  $104^{\circ}$  and  $106^{\circ}$  is also shown. What does not show was the change in the child's general appearance and attitude. Within one week after treatment she had changed from a listless lackadaisical child with a poor appetite, to a lively, active one, bright and interested in her surroundings, and with a ravenous appetite. She was given a second treatment in the ninth week after the first, because al-

TABLE II

NO	NO ADJ CARDIAC DIAGNOSIS	SIGNS OF ACTIVE CARDITIS	DURATION THERAPY	DURATION OF CLIN- ICAL SIGNS OF CARDITIS	DISCHARGE DIAGNOSIS
1	a Rheum* b Carditis c ST d E & F	Tachycardia, develop- ment of systolic and diastolic mur- murs under obser- vation	8 days	End of therapy, rate slower, murmurs softer 10 days lat- er, rate 78, SA present	a Rheum b MI & S c SA d Ila
2	a Rheum b EH, MI Carditis c ST d I	Fever on admission, tachycardia, gallop Quality of systolic presence of diast- olic	8 days	End of therapy, rate 80, no gallop, SA present 4 days later, diastolic mur- mur no longer heard	a Rheum b FH, MI c SA d I
3	a Rheum b Carditis c ST d E & F	Tachycardia, gallop development of sys- tolic murmur	8 days	After 1 fever, no gal- lop, rate 100. End of therapy, rate 88 murmur less loud 10 days later, no murmur	a Rheum b -- c SA d F
14	a Rheum b Carditis c ST d E & F	Fever, tachycardia, gallop. Develop- ment of diastolic murmur, prolonged P R interval	17 days	5 days after begin- ning of therapy rate 80-90. No gal- lop. P R interval normal before end of therapy	a Rheum b -- c SA d E & F
16	a Rheum b EH, MI Carditis c NSR d I	Gallop, high pitched shrill systolic Pres- ence of diastolic	8 days	5 days after begin- ning of therapy, no gallop, systolic soft and low pitched No diastolic heard	a Rheum b EH, MI c NSR d I

\*Diagnoses were made according to the criteria for the classification and diagnosis of heart disease of the Heart Committee of the New York Tuberculosis and Health Association. Abbreviations used are: EH, enlarged heart; MI, mitral insufficiency; MS, mitral stenosis; NSR, normal sinus rhythm; SA, sinus arrhythmia; ST, sinus tachycardia; E, possible heart disease; F, potential heart disease; I, functionally able to carry on normal activity; Ila, functionally able to carry on with slightly limited activity.

though her general condition continued to be good her temperature on two occasions was above 100, and her sleeping pulse rate began to be slightly elevated. Following this her progress was steadily uphill.

M. M. (Fig. 7) was twelve years old when admitted in February, 1934. She had had repeated attacks of chorea since the age of four and one half years but no other rheumatic history. She had been followed by us since September, 1931, when she was treated with fever therapy for a moderate chorea. Since that

time she had had two very mild attacks of chorea. Her heart had never been enlarged but there had been an apical systolic murmur, transmitted slightly, present from the time of her first admission, and a diastolic murmur had first been heard in December, 1932. For two months previous to admission the mother had noticed tachycardia; dyspnea on climbing one flight of stairs had developed and the child had been complaining of frequent precordial pain. Two weeks before admission she had had a sore throat and had been worse since that time. On admission her temperature was  $100.4^{\circ}$ , heart rate was 130-140, there was a marked presystolic gallop, an apical systolic murmur with a high pitched shrill musical quality and a short middiastolic; no sinus arrhythmia was present. In view of the observed tachycardia and precordial pain of two months' duration, she was treated promptly without long observation in the hospital. The first treatment was for four hours at  $105^{\circ}$  to  $106^{\circ}$ , and the second, two weeks after the first, was for three hours at  $106^{\circ}$ . Following the first treatment the signs of activity decreased but they did not completely disappear until after the second. The most striking immediate change was the disappearance of the shrill musical quality of the apical systolic murmur. There was also the same change in the general appearance and behavior that was shown in the first case.

#### CONCLUSIONS

Fever, by whatever means produced, is a satisfactory method of treatment of chorea, in that the duration of the attacks is thereby appreciably shortened.

The presence of subacute carditis or of inactive rheumatic heart disease is not a contraindication to the use of fever therapy in treating chorea.

The subsequent uphill course of patients with subacute rheumatic carditis who received fever therapy suggests that fever may be of benefit to such patients. We believe that further investigation is warranted.

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#### DISCUSSION

DR PHILIP S. HENCH, ROCHESTER, MINN.—There is a frame of mind among certain physicians that all "machine medicine" is tinged with quackery, that even if the *deus ex machina* is a benign god, he should nevertheless be considered an unorthodox deity. Such physicians look with a suspicion, not always unjustified, upon anybody who uses machines in therapy. Fever therapy has had its origin in the laboratories of scientists of national and international repute. Its clinical utilization has so far been entrusted almost exclusively to physicians associated with large clinics and research institutions, men familiar with accurate methods for such investigations. Nevertheless, fever therapy is still enjoying its "honeymoon period" and reports thereon are still apt to be a bit enthusiastic.

The report of Drs. Sutton and Dodge is expressed in conservative terms. Their results are in general agreement with those reported at the Fifth Fever Therapy Conference in Dayton last May. Of a total of twenty-five cases of Sydenham's chorea treated by Hefke, Bierman, Schnabel, Schmidt, and Metz, about twenty cases (80 per cent) were apparently notably benefited even though several had previously not been relieved by reactions to typhoid vaccine given intravenously. A number of patients had carditis or mitral endocarditis; this was not considered a contraindication to fever therapy. Usually four or five treatments of three hours each at  $105^{\circ}$  to  $106^{\circ}$  F. were given.

At the Mayo Clinic under the clinical observation of Drs. Helmholtz and Amberg ten cases of Sydenham's chorea have been treated with fever therapy by Drs. Desjardins and Popp. The age of the patients averaged ten years (youngest seven, oldest fifteen years). The duration of infection ranged from one week to four years. From four to twelve (average six) fever sessions of three hours each were given. The patients have been observed for from six to fourteen months since treatment. One was remarkably relieved, eight were definitely benefited. One to whom inadequate fever was given was not helped. A temperature of 104° to 107° F., not lower, is advocated.

From these various preliminary experiences it seems proper to agree with Drs. Sutton and Dodge that fever therapy is a helpful and promising form of therapy for chorea.

DR. JOHN R. NOTE, BOSTON, MASS.—It seems from the results presented that patients with chorea are helped by the use of typhoid vaccine or by artificial fever. In considering such results, however, it is necessary to keep in mind that chorea is a symptom complex and not a disease entity, the diagnosis of which is frequently difficult and not uncommonly open to question. More difficult still is the determination of cessation of the disease. This in our experience has been a repeated issue for discussion in following the clinical course of many patients. In any condition where there may be wide differences in the criteria of complete recovery the results of the use of any therapeutic agent must be open to considerable individual interpretation. Typhoid vaccine treatment has not been used on an extensive scale at the House of the Good Samaritan. In our limited experience with this therapeutic agent in this condition, we noted that in all instances the choreiform movements were definitely decreased, but unfortunately only temporarily so. In most instances they were not given quite such intensive treatment as that used by Drs. Sutton and Dodge. Since our results were neither complete nor permanent we have not felt that such severe treatment was indicated in the usual case of chorea. In the severe and exhaustive form of the disease typhoid vaccine may be an indicated form of therapy.

Dr. T. D. Jones and Dr. E. F. Bland have recently completed the analysis of a follow-up study of 1,000 consecutive chorea and rheumatic fever patients at the House of the Good Samaritan who have been followed over an average period of eight years since the onset of their disease. In this study chorea was taken as one manifestation of rheumatic fever and analyzed as such, and all other manifestations of rheumatic fever were considered other evidence of rheumatic fever. There were 482 patients who had chorea as one of the manifestations of rheumatic fever whereas 518 patients had other evidences of rheumatic fever without chorea. Of the 482 patients who exhibited chorea, 54 per cent had clinical evidence of heart damage, in contrast to this, of the 518 patients who had only other manifestations of rheumatic fever, heart damage was present in 86 per cent of the cases. When the group of 482 patients who had chorea as a manifestation of rheumatic fever is further subdivided into those who had chorea as the only manifestation of rheumatic fever and those who had in addition to chorea other evidence of rheumatic fever it was found that the incidence of clinically demonstrable heart disease in the former group of "pure" chorea was only 3 per cent. The latter group of patients having chorea with other evidence of rheumatic fever had an incidence of 73 per cent of heart disease, clinically detectable. Therefore, instead of being a grave manifestation of rheumatic fever, chorea in itself is seen to be a mild manifestation. And patients with chorea alone show no special predilection to heart disease unless other evidence of rheumatic fever occurs. On the other hand any patient with chorea and with other evidence of rheumatic fever, either laboratory or clinical should be treated as any other individual with active rheumatic fever.

A year ago Drs. Jones and Bland reported on the use of single intravenous doses of typhoid paratyphoid vaccine in 12 rheumatic fever subjects and noted definite recurrences in six instances. If this is even an uncommon occurrence it is a contraindication for the use of vaccine therapy where there may be evidence of rheumatic fever other than chorea.

DR. HOMER F. SWIFT, NEW YORK, N. Y.—When should fever therapy be applied in a case of rheumatic fever? In former times "hyperpyrexia rheumatica" was one of the most dreaded and fatal complications of acute rheumatic fever. It is conceivable that if this type of therapy were applied to a patient at a time when the heat regulating mechanism was very

unstable, it might place him in a state of uncontrollable hyperpyrexia, when later it might not. So many rheumatic children have evidence of low grade fever and persisting infection, that any relatively safe measure which promises aid in eliminating these symptoms should receive a thorough trial. Our own experience with fever therapy has been too limited to permit of seasoned judgment.

DR. E. STERLING NICHOL, MIAMI, FLA.—In discussing fever therapy in patients with active carditis one should differentiate between fever therapy produced with foreign protein and that induced by heat. When I was an interne on the service of Dr. Joseph L. Miller in Chicago twelve years ago, we gave intravenous typhoid vaccine to all patients with rheumatic fever *without* carditis. At that time we felt that cases with active carditis did not tolerate foreign protein therapy well. Is your experience with foreign protein in active carditis similar to that with fever therapy induced by the "hot-box"? The latter may be safer than the use of foreign protein.

DR. JOHN WYCKOFF, NEW YORK, N. Y.—This paper makes three propositions: the treatment of Sydenham's chorea with foreign protein fever, and with artificial fever, and the treatment of rheumatic carditis with artificial fever.

In our section of the country we do not feel that chorea is such a simple manifestation of rheumatic fever as Dr. Mote seems to think. The course of chorea can be tremendously shortened and the symptoms almost immediately alleviated by the use of foreign protein. As a result of such treatment chorea patients have practically disappeared from the services of the Bellevue Hospital.

With artificial fever therapy the results are even better. The temperature can be controlled. If the patients get too excited or ill the temperature can immediately be reduced. In every way it seems to be a more effective method of treatment.

We are most interested in the effects of fever therapy on patients with definite, active rheumatic carditis. The number of such cases treated by Drs. Sutton and Dodge is very small, but in practically every one of their sixteen patients, a definite change in the course of the disease is reported. Enough time has not elapsed for one to say that such cases will show fewer relapses than those which have been allowed to run the natural course of the disease.

DR. SUTTON (closing).—Though chorea seems to be the least serious of the rheumatic manifestations, it does lead eventually to cardiac damage, one reason for believing that rather heroic treatment is justified. Chorea keeps children out of school for weeks and months and is not a pleasant disease to have. Some children return asking for more treatment. They would rather have the foreign protein reaction and then be able to go back to school than to lie in bed for weeks.

In using foreign proteins we aimed for a high fever and not just for a temperature of 102°. Although we have treated 300 or 400 cases of chorea with typhoid-paratyphoid vaccine we have never noted any bad effect and never induced an attack of carditis or polyarthritis.

For the treatment of carditis we selected cases of low grade rather than acute activity. We did not treat children who had histories of congestive heart failure. We treated one acutely ill child who had chorea and also a severe acute carditis which had followed an acute polyarthritis just before her admission to the hospital. Although she was not definitely or immediately benefited by the two fever treatments she stood them well, even though during the first hour of fever her pulse rate became very rapid. Two weeks later when we gave her a second treatment she had no dyspnea and could lie flat in bed. Her pulse did not become so rapid. Now she is much better; indeed she is the picture of health.

In using hot packs for the production of fever we had one fatal result. With typhoid-paratyphoid vaccine we have had no fatality. In our first report we included the case of one child whose temperature was raised by means of a blanket pack; her temperature got out of control and went up to 109°. Clinically she had shown no evidence of activity, but at autopsy there was a definite verrucose endocarditis of the mitral valve and an old endocarditis of the aortic valve. Though the temperature was brought down she died the next day.

## A THEORY CONCERNING THE MECHANISM AND THE SIGNIFICANCE OF THE ALLERGIC RESPONSE

WARREN T. VAUGHAN, M.D. RICHMOND, VA

IN THE three hundred years since Harvey introduced the experimental method this has become the most useful procedure in the study of normal and pathologic physiology. Given an abnormality such as albuminuria one can study the anatomic changes in the organ responsible for the production of urine, establish functional and organic changes in the blood and tissues and their relationship with the abnormal renal findings, and produce organic changes in animals sufficiently like those observed in man to facilitate further study of the problem. It is true that the nephritic changes produced in animals are not identical to those observed in man, but the parallelism is clear enough to permit studies which have widened our knowledge of renal disease.

The same may be said of many other functional and organic pathologic states. Their reproduction or near reproduction in animals has facilitated research and has broadened our understanding.

Curiously, in one group of maladies the experimental method has anticipated itself, causing much confusion. The customary procedure has been to select a *problem for study*, to devise a *program for the production of an analogous lesion* in experimental animals to accomplish this and then to proceed with those studies which cannot be suitably carried out in man himself.

But in the late nineties the reverse situation manifested itself. Diphtheria antitoxin had been discovered by Von Behring, confirmed by Roux, and was being produced in volume by the pharmaceutical supply houses. Guinea pigs used for potency tests reacted in curious ways. Once having been used, they were likely to die suddenly and unaccountably if used a second time. Richet and Heirourt, later Portier and Richet, observed a similar curious phenomenon following repeated parenteral injections into dogs of sea anemone extract. Richet described a new phenomenon, not comparable with anything previously recognized as disease in man. He established two fundamental points which remain unquestioned: (a) that a foreign organic substance though harmless in a given dose on first injection might be most harmful even fatal, following a second injection in the same dosage and (b) that an interval of several days must elapse between the first and second injections before the substance becomes poisonous.

Except for the few curious reactions in human beings following the injection of diphtheria antitoxin that were then making their appearance, there was no analogy in human disease.

It was not until 1906 four years after Richet had propounded his theory, that Wolff Eisner suggested that hay fever might be an anaphylactic phenomenon. It was not until nine years after Richet's work that Noon and Freeman conclusively demonstrated the accuracy of this suggestion. There was a lapse of

eight years before Meltzer, basing his suggestion on Auer's observation of broncho-constriction, first suggested (1910) that asthma might be an anaphylactic disease. Not until 1921 did Duke demonstrate the anaphylactic basis of gastrointestinal allergy. Thirteen years elapsed before Rohrer suggested that migraine might be associated with this curious experimental phenomenon and a full twenty-five before Vaughan proved the association by allergic methods. Twenty years elapsed before Vaughan demonstrated a similar relationship in certain cases of mucous colitis.

We may say, however, that after 1910 investigators were beginning to realize that a group of clinical diseases might be explained on the basis of the knowledge that was accumulating concerning experimental anaphylaxis or protein sensitization. Gradually new diseases were being added to the list, diseases which had no apparent intimate association with one another. We commenced to speak of the allergic diseases.

The next phase was a rather acrimonious discussion concerning the mechanism of the phenomenon and a similar debate regarding the similarity or identity of clinical allergy as observed in human beings and experimental anaphylaxis as seen in animals. Both debates continue, but with decreasing momentum. With regard to the second, which holds our major present interest, there is still wide divergence of opinion. There are many who insist that points of dissimilarity between clinical allergy and anaphylaxis render an actual identity impossible. The principal points made are: (1) that anaphylaxis is an antigen-antibody phenomenon in which antibodies and precipitins can readily be demonstrated in the blood, while such substances are not present in the blood of allergic human beings; (2) that animals may be sensitized with ease while it is almost impossible to render human beings artificially hypersensitive; (3) that though protein sensitization can be transmitted passively from mother to offspring, active hereditary transmission cannot be accomplished in animals, while human allergy is predominantly an hereditary malady.<sup>1</sup>

These are points of difference which I have always felt were nonfundamental and that further research would eventually reconcile.

If the sequence had been reversed, if there had existed a certain clearly recognizable disease complex which we shall call allergy, and animal research had followed rather than preceded a recognition of the clinical entity, the studies of experimental anaphylaxis would have been accepted with little or no question, as observations elucidating the phenomena of allergy. There are points of difference between nephritis in human beings and experimental uranium nephritis, but, those differences being recognized, investigators do not quibble as to whether the experimental disease is analogous, within the limits of the study. But in allergy for some curious reason, since the experimental observations antedated the clinical application, more attention has been bestowed upon the discrepancies than upon the fundamental similarity.

It shall be my purpose in this communication to demonstrate the basic identity of the two; to present the various apparently unrelated allergic manifestations as an integrated symptom-complex; and to explain how these diverse symptoms actually represent a rational coordinated purposeful response. This

explanation is predicated upon the assumption that the allergic response, no matter what its clinical manifestation of the minute, is as much a disease entity as is hypertension, with its associated arteriosclerosis and cardiovascular pathology. The difficulty in the past has been that, since no single organ can be identified either functionally or structurally as the primary seat of the malady, it has been difficult to select a point of departure for study.

Our theory can be most clearly presented by a series of dogmatic statements, each of which is followed by clinical or experimental observations.

#### I ALL PERSONS ARE POTENTIALLY ALLERGIC

1 *There is no fundamental difference between clinical allergy and experimental anaphylaxis*—The three chief points of contention made by those who deny a basic identity have been mentioned. The absence of antibodies in the blood of human allergies, at least so far as our present methods can demonstrate, is granted. That there is some sort of antibody in the blood is demonstrated by the phenomenon of passive transfer by the Prausnitz-Kustner method. The designation "reagin" has been proposed<sup>1</sup> in recognition of their presence and to emphasize that they are probably different from the antibodies of laboratory animals. In other words there is something in the blood with which we can produce passive transfer in human beings. It cannot be identified by precipitin tests or by complement fixation reactions.

For a time it was believed that precipitin represented the antibody, but there is now some doubt regarding this, and many believe that while precipitins accompany antibodies, they are not the antibodies themselves.

Doerr and Russ found that the ability of serum to passively transfer anaphylaxis is proportionate to its precipitin content. However, as stated by Seegal,<sup>2</sup> it is sometimes possible to transfer hypersensitiveness with serum in which no precipitin can be demonstrated. "It may therefore be that the antibody responsible for the transfer of anaphylaxis is not a precipitin, but some substance which increases in amount in the serum of immunized animals coincidentally with the precipitin." Matsumoto<sup>3</sup> has observed that in guinea pigs precipitins may persist in organs and tissues long after they have completely disappeared from the blood. According to the prevailing hypothesis of anaphylaxis, the reaction is cellular rather than humoral, and the presence of tissue antibodies is therefore of much greater importance than that of antibodies in the blood serum. Until recently the demonstration of tissue antibodies has been difficult or impossible. Khorazo<sup>4</sup> however has recently prepared practically pure tissue juice by breaking up the individual cells under high pressure. Seegal and Seegal<sup>2</sup> have demonstrated cytoplasmic antibodies in such tissues. They found, further, that typhoid agglutinin existed in the tissues in from two to four times its concentration in the blood. Occasionally they even demonstrated agglutinins in the tissues when none at all were demonstrable in the blood. Therefore, whether antibodies and precipitins are identical or not, the fact that these are not present in human allergies in no way invalidates an identity between clinical allergy and experimental anaphylaxis.



The second point, that it is difficult or impossible to sensitize human beings, will be discussed in section 7.

With regard to the third point, the recent investigations of Ratner<sup>4</sup> have shown that it is possible to transmit active sensitization in animals from mother to offspring.

2. *The allergic response is not limited to protein but may be made to a large variety of nonprotein substances.* The reaction to protein is the most dramatic, was the first type observed and is the most easily studied, since it is so easily reproduced in animals.

The first nonprotein substances added were those drugs which sometimes caused symptoms indistinguishable from allergy when administered enterally or parenterally. The early suggestion of Wolff-Eisner, the investigations of Obermeyer and Pick and of Jadassohn, and the more recent conclusive work of Landsteiner on haptenes have provided a rationale for the interpretation of drug allergy, in terms of protein sensitization.

Typical allergic symptoms, due to heat, cold, light and effort, appear to bear no connection with protein sensitization. This is likewise true of contact allergy to nonnitrogenous substances, although at present we know nothing concerning the possibility of a union of these substances with the proteins of the superficial tissue cells.

The possibility, as developed principally by Avery, that certain carbohydrates may sensitize although they cannot shock unless conjugated with specific proteins should be mentioned.

The present theory does not require that protein be implicated as the basis of all allergic responses. At the same time such an eventuality would not invalidate the hypothesis.

3. *There is no fundamental difference between the allergic and the so-called nonallergic individual. The response of the allergic person differs from the nonallergic in degree, not in kind.* Raekemann<sup>5</sup> (1930) wrote, "It seems proper to assume that hypersensitiveness is acquired in most cases and probably in all. What we call allergy may well be nothing more than anaphylaxis in man. . . . The production of antibodies in general is a normal function. Allergy is a reaction of a particular kind which is characterized by the easy formation of cellular antibodies in great abundance. . . . When the individual has several different allergic symptoms at one time; when his family history shows allergy in his antecedents, in his children or perhaps in both; when his symptoms are severe or when his skin tests are large, that individual may be assumed to have an easy tendency to develop hypersensitiveness. He is allergic only in this sense."

Vaughan<sup>6</sup> (1933), in a study of a series of cases of major allergy and minor allergy, observed no fundamental difference between either of these groups or the so-called nonallergic group. He concluded, "Allergy is not a pathologic state. It is a pathologic exaggeration of a normal physiologic response."

Raekemann<sup>7</sup> (1933) found no fundamental difference in the immunologic response, the development of typhoid agglutinins and other agglutinins, in allergies and in nonallergies.

Rackemann and myself were so far as I have been able to determine, the first to express the belief that there is no fundamental difference between the allergic individual and the nonallergic. All gradations between the two groups exist.

4 *Clinical allergy is much more common than has been hitherto suspected.* The surveys of Cooke and Vanderveer<sup>8</sup> and of Piness and Miller<sup>9</sup> would indicate that not over 10 per cent of the population is allergic.

I, however, found in a survey of an entire community<sup>10</sup> that while the frankly allergic did comprise about 10 per cent, there was an additional 50 per cent who had had some clear cut allergic manifestation at some time in their past experience. I called these *minor allergies* in contrast to the first group of *major allergies*. There was, in general, a distinct difference between the two groups, in that the minor allergies were able almost without exception to designate the cause of their symptoms while the major allergies could not do so. The minor allergic or fortunate allergic was sensitized to some substance with which he came into only occasional contact and which he was therefore easily able to recognize. The major or unfortunate allergic was, as is observed in allergic practice, sensitized to substances with which he came into frequent or constant contact and therefore could not recognize them. More than 50 per cent suffered from minor allergy but all questionable cases were classed as nonallergic. The total figure is therefore conservative.

These statistical findings are confirmed in a measure by the observations of Rowe<sup>11</sup> who found that 35 per cent of a homogenous student population gave positive allergic histories. Of Hinkley<sup>12</sup> who, using only three allergens, found positive skin reactions in 38 per cent of a group of general medical ward patients, and of Rackemann and Simon<sup>13</sup> who using nine test allergens found positive reactions in 50 per cent of presumably nonallergic persons.

5 *The question should no longer be, "Why do some persons become allergic?" but rather, "Why are not all persons allergic?"* If over 60 per cent of the population, the majority, have or have had some form of allergy, severe or mild, then allergy becomes the rule. The so called normal case becomes an exception, and it is the latter which then requires explanation.

I<sup>14</sup> have stated (1934), "The probability is that the development of sensitization to foreign substances is almost a normal physiologic function, and that if all of us were to live long enough 100 per cent of the population would at least develop minor allergy."

6 *All persons possess the potentiality of becoming allergic, the susceptibility varying only in degree. It seems probable that in any population the degree of susceptibility to the development of allergy varies from 100 per cent to zero per cent according to a mathematical formula, depending upon individual variances in susceptibility.*

Spain and his collaborators<sup>15</sup> find that under equal conditions of exposure the percentage of adult human beings susceptible to poison ivy or poison oak varies as the logarithm of the concentration of the irritant applied. This is similar to the response to drugs and other physiologic stimuli in general in agreement with the Weber Fechner Law. We may simplify these conclusions somewhat as follows. A given percentage of persons will give positive reactions

to patch tests with 1/1,000 dilution of ivy extract. If those who failed to react are now tested with 1/100 dilution, the same percentage will be found to react. If those who fail to react to the 1/100 are tested with 1/10 concentration, the same percentage will be found to react.

This could be interpreted as indicating that, provided the concentration could be made high enough, all people would react to *Rhus toxicodendron*. All could be made susceptible but with varying degrees of resistance thereto. There is evidence that this is true of other forms of contact dermatitis.

Stewart and Cormia<sup>16</sup> produced nickel dermatitis in guinea pigs, constantly, following cutaneous application. They found that the lesions were similar in every respect to those described in man as nickel dermatitis. The intensity of the reaction was in direct proportion to the concentration of the solution used.

From this we could conclude that, given sufficient concentration and enough exposure, nickel dermatitis can be produced in 100 per cent of instances. This corresponds with the early observation of Walthard (1926) that from 41 to 100 per cent of workers in the Swiss nickel industry developed nickel dermatitis. There was an incubation period in these cases of from fourteen to twenty-one days. Steiner observed positive patch reactions to nickel in 50 per cent of cases of neurodermatitis.

Schittenhelm and Stockinger state that all workers constantly exposed to nickel salts eventually develop eczema. Apparently the degree and concentration of exposure play a great part. Jadassohn remarks that nickel eczema is common in large factories and only occasionally observed in small nickel shops.

These findings correspond to those noted by Spain et al. in ivy poisoning. Bloch<sup>17</sup> has made observations on primula sensitization bearing out the same point. Normal, nonallergic persons were found rarely sensitive to primula, but if the concentration of the antigen was increased, he was able to overcome this constitutional resistance, producing primula sensitization in nonallergic individuals.

Stewart and Cormia remark that sensitivity to chemicals is more easily developed when the exposed individual has a hyperirritable type of skin which develops multiple sensitivity on only occasional contacts.

These observations all tend to confirm the premise which I have already stated, that in dermatitis, even nonorganic dermatitis such as to nickel; in contact dermatitis of the rhus type; and probably also in frank allergy or atopy, it is possible to sensitize 100 per cent of individuals, depending upon the degree and length of exposure. Some individuals become sensitized easily, while others become allergic only in spite of great resistance. There are all gradations between the two extremes, and these gradations can probably be expressed mathematically.

In making this statement I do not mean to imply that there are not other as yet ill understood factors which determine the substance to which one will become allergic, given comparable degrees of exposure.

While the experimental findings deal with nonprotein sensitization the cumulative evidence strongly suggests a similar situation with regard to protein sensitization.

7 *The nature of the allergen plays a part* It has been claimed that there is a fundamental difference between experimental anaphylaxis and human allergy in that human beings can be sensitized to allergens, only with greatest difficulty or not at all, that when sensitization does occur it appears to be entirely spontaneous and probably associated with hereditary predisposition

It is quite true that Brimmer<sup>18</sup> found that he could not easily sensitize individuals to pollen extract or orris root even after repeated injections On the other hand he sensitized to ascaris extract with no difficulty

Jones and Mote<sup>19</sup> found no difficulty in sensitizing human beings to rabbit serum Simon and Rackemann<sup>20</sup> experienced similar success with guinea pig serum, whether administered through the skin or applied to the nasal mucosa As a matter of fact the ease with which human beings may be sensitized to foreign serum has been in evidence for many years The only trouble has been that we have not realized it The majority of persons who receive therapeutic horse serum develop serum sickness, evidencing sensitization Hooker<sup>21</sup> has shown that toxins given simultaneously increase the tendency Twenty seven per cent of cases receiving toxin antitoxin subsequently developed positive skin reactions to horse serum Gordon and Creswell<sup>22</sup> found that 74 per cent of individuals receiving serum who had previously received toxin antitoxin, gave serum reactions Forty three per cent of those who had previously received therapeutic serum (not toxin antitoxin) reacted after a subsequent serum injection Tuft<sup>23</sup> found that 28 per cent of children receiving diphtheria toxin antitoxin became allergic to horse serum

These observations support my contention<sup>10-14</sup> that the average person or animal may be sensitized more easily to a foreign protein or substance with which he establishes only occasional contact than to one in which the contact is relatively more constant

It is difficult to sensitize man to foods which he eats frequently, to feathers, orris root, pollens, house dust—those things to which he is relatively frequently or constantly exposed Only the 10 per cent who are most highly susceptible to the development of the allergic response become sensitized to common allergens Those less highly susceptible are more likely to react to proteins which are much more foreign to their economy This forms the basis of differentiation between the major allergic and the minor allergic

It is interesting to conjecture that, had the early experiments leading to the discoveries in anaphylaxis been made with substances to which the animals were constantly exposed, the results would have been far different If guinea pigs eating celery had been injected with celery protein there probably would have been as much difficulty in sensitizing them as occurs when human beings are injected with orris root or pollen extracts Indeed, it has already been shown that it is as difficult to sensitize animals to pollens as it is to sensitize human beings If dogs eating beef had been injected with beef extract rather than the extract of sea anemone with which they had never experienced former contact the results might have been different

In this way the nature of the allergen plays a part in determining sensitization Let us assume that among human beings there are all degrees of suscepti-

bility to the development of allergy, from those highly insusceptible to the last 10 per cent, who are very susceptible. The intermediate group, represented in my series by the so-called minor allergies, becomes allergic only to those substances with which they establish occasional contact. In the case of proteins these are unusual proteins, proteins to which the individual has not become acclimated. Among the foods this includes such as onions, cabbage, tomato, strawberry, shell fish, and the like.

The middle group, the minor allergies, do not appear to become sensitized to frequent contactants. This is analogous to the observation of Wells<sup>2</sup> who found that young guinea pigs bred from mothers fed on oats could be shown to be highly sensitive to oats, when this food was eliminated from their diet. If they were fed on oats they acquired immunity thereto. But those with an extreme susceptibility to allergy become allergic even to those foods with which they come in frequent contact. Indeed, they are especially likely to do so. This does not mean that they do not also become allergic to the occasional contactant. As a matter of fact in my experience they do so in the same degree and frequency as do the minor allergies. The difference is that in addition to becoming allergic to occasional contactants they also become allergic to frequent contactants.

We do not yet know why a person will develop sensitization to one allergen and not to another, assuming the same frequency or intensity of exposure. There is much that we can assume. We can assume an abnormal permeability of the gastrointestinal tract at some particular time which will predispose toward sensitization. We can assume intercurrent illness. We can accept the evidence that certain foreign substances, such for example as ascaris, are much more likely to produce sensitization than are others, possibly on account of some curious factor in their chemical make-up. We can assume any number of nonspecific factors which might predispose toward sensitization. We can recognize the observations of Walzer<sup>25</sup> and his collaborators, demonstrating that food allergen is normally absorbed through the gastrointestinal tract and transported by the blood in a state still sufficiently like its original so that it can be identified by biologic test (passive transfer). The fact remains that there is a difference between parenteral injection of the foreign protein and the normal digestion, and absorption of the same. Even though it may not be broken down into its constituent amino acids before absorption and even though the foreign protein may in some degree maintain its identity following absorption, the fact remains that only occasionally does it cause sensitization. Digestion must therefore have produced sufficient changes so that there is a difference between the antigenic capacity of parenterally introduced native protein and the same protein after digestion.

In any event, there is much that we do not know as to why one becomes allergic to one substance and not to another. The evidence so far would indicate that a protein or other substance to which one has not been customarily exposed is more likely to produce sensitization. *Those who are unusually susceptible, the more intense or the more likely is one to develop sensitization.* *ure, the*

8. *Inheritance probably plays a part in the all*  
scarcely needs further elucidation. The evidence amas.

*This*

ance of the allergic tendency appears incontrovertible. Undoubtedly children of parents both of whom are allergic are more likely to manifest allergic symptoms in greater proportion and at an earlier age than children of parents only one of whom is allergic and even more so than children, neither of whose parents is allergic.

Nevertheless it is of interest that I<sup>6</sup> have observed in a series of 100 cases studied for minor allergy that the allergic inheritance is equally heavy in those classified as nonallergic as in those classed as minor allergies. It is only in the major allergies that the inheritance is more pronounced.

*Discussion*—I am not alone in the belief that experimental anaphylaxis and clinical allergy represent a fundamentally identical phenomenon. Many writers have expressed the same idea. Broufenbrenner<sup>36</sup> has very recently presented a most comprehensive and convincing review of the subject in support of his belief that there is no fundamental difference. Zinsser has written, in his volume *Resistance to Infectious Diseases*, as follows:

The obvious analogies of many human conditions such as asthma, hay fever, drug and bacterial idiosyncrasies to protein anaphylaxis in animals led early in the development of this subject, to attempts to elucidate the relationships by clinical and experimental investigations. The difficulties of such researches were, however, many and it became advisable as a trillise for speculation and experiment to construct tentative classifications of the different forms of hypersensitiveness, based chiefly upon the antigenic nature of the responsible substances and the demonstrability of an antibody mechanism by success or failure of passive transfer. Doerr and Coca particularly established divisions of the subject which we then held as too rigidly conceived. Coca laid particular stress upon the separations he believed enforced by the apparently nonantigenic nature of some of the most characteristically allergenic substances and by an exaggeration (in our opinion) of the importance of heredity as opposed to sensitization by previous contact. He was hampered in his reasoning by gaps in our knowledge—bridged, since then, by the discovery of partial antigens by the demonstration of intra-uterine and intestinal spontaneous sensitization of man and by the recent recognition of the importance of homologous as opposed to heterologous passive transmission. At the present time most workers who have given the subject serious thought agree in fundamental principles, though in regard to serum sickness and a few other conditions diversity of opinion still prevails.

Evidence has been presented in the preceding pages supporting the contention of Rackemann and myself that the allergic and nonallergic individuals are fundamentally alike in their reactive capacities.

If these two premises are correct it becomes obvious that a theory which attempts to explain the manner of the allergic response should correlate the findings of experimental anaphylaxis with those observed in clinical allergy and with normal physiologic responses. An adequate theory must recognize a denominator common to all three groups. Such a theory must therefore be broader and more generally inclusive than those which have been heretofore proposed. The theory must recognize a mechanism common to all three groups in which the group differentiation is not based on fundamentally different reactive processes.

Allergy must be studied from a much broader point of view than heretofore. I believe that allergy does actually represent a much broader group of phenomena than that of protein sensitization to which it was at first assigned. Just as the earliest experiments in protein anaphylaxis were the most spectacular and

therefore received chief attention, so also the *clinical* observations on protein sensitization were the most spectacular in this field. But, by now, nearly all will agree, I am sure, that clinical allergy involves much more than protein sensitization.

Since the earliest work dealt with proteins, and since the early theories were expressed entirely in terms of protein poisoning, it has been very difficult for many to overcome the mental hazard involved in the acceptance of a conception of nonprotein allergy.

In the present theory I go even farther in this direction by explaining phenomena such as physical allergy and psychogenic allergic reactions which have not hitherto been adequately fitted into the picture of the allergic response, and presenting the entire group of phenomena as a variant of the normal processes of immunity or protective adjustment toward one's environment.

In the years that have elapsed since the development of the original theories explanatory of anaphylaxis, there has been developed such a maze of conflicting and confirmatory laboratory observations that a clear interpretive perspective has become difficult.

I feel, however, that enough experimental material has been accumulated to justify a philosophic approach based upon these observations. The theory herein is presented as such but with emphasis placed on the fact that it is based on the cumulative experimental and clinical researches of the last quarter century.

## II. THE ALLERGIC RESPONSE IS BASED ON AN INTEGRATED PURPOSEFUL PHENOMENON

9. *A dominant requirement of all life is that it maintain adequate adjustment to its environment.* Primordial life, whether purely chemical or particulate, as exemplified by the amoeba or possibly the phage, depended upon a narrow margin of chemical and physical environmental factors for its continued existence.

The simple cell aggregates worked out communal methods of protection against deleterious environmental influences. The simplest and undoubtedly one of the earliest was in the protective covering of specially differentiated cells.

In man we find an intricate but withal correlated mechanism of protection against extrinsic factors. This protective system includes the skin; mucous membranes; ciliated epithelium; hairs on the body, in the nostrils, in the ears; the turbinates, uvula and epiglottis; the digestive juices; leucocytes; opsonins and antibodies. Among the more obviously protective reflexes we might mention blinking, the pupillary reaction to light, sneezing, coughing, the gag reflex, vomiting, diarrhea, smooth muscle spasm and the coordinated protective reflexes of voluntary muscles.

Deleterious influences against which the body must protect itself include physical factors such as trauma, extreme changes of temperature, intense light, electricity, ultraviolet, x-ray, radium, short wave radio, chemical factors such as acid, alkali, drugs, arsenic, paraphenylenediamine, and biologic factors such as infectious agents, toxins and foreign proteins.

10. *A certain degree of acclimatization is possible by which increased tolerance for deleterious extrinsic factors may be established.* While there is some degree of natural acclimatization to atmospheric factors, to poisons, repeated

low grade injury (callouses) and other extrinsic factors, nevertheless this environmental adjustment has rather narrow limits. The morphine addict acquires tremendous tolerance for the drug but he may still be poisoned with morphine. Our continued existence on this planet depends upon the continuation of an optimal temperature range which is not great. It seems quite probable that many of the species of plants and animals which have ceased to exist have done so because they were unable to acclimate themselves to deleterious environmental influences.

One of the simplest examples of normal increased tolerance is that of adjustment to changes of temperature. The first cool spell in the winter seems colder than a much more pronounced temperature drop later in the season, when one has become adjusted. The first hot days of summer are more enervating than those of midsummer. A person from the temperate zone who moves to the tropics finds that he cannot accomplish as much productive work as formerly until after he has been a resident in the tropics for some months and has become acclimated. This question of acclimatization is, as has been brought out by Duke,<sup>6</sup> an important one in the production of physical allergy or hypersensitivity to heat or cold.

The simplest heat sensitive individual according to Duke is the one who has allergic symptoms only on extremely hot days. Slightly different is the person who has symptoms earlier in the summer but not in midsummer because by then he has become adjusted. Still different and more difficult to recognize is the patient who tolerates midsummer temperature but not a sudden increase, even though the increase be to a temperature which is not as high as that of midsummer. This is the person who experiences symptoms after leaving an air conditioned, cooled cinema. The reverse applies to the cold sensitive individual. Where the hypersensitivity is manifested more to changes of temperature than to the actual degree of temperature, we find curious situations. Thus the heat sensitive individual may have symptoms only in midwinter when going into a heated house. He cannot tolerate a change from 20 above zero to 70 even though during the summer he had no symptoms at 80. In the summer he had become acclimated. In the winter the change was too sudden for acclimatization. In the same way the cold sensitive individual according to Duke may have symptoms only in the summer time. He adjusts himself to winter temperatures but when in the summer he enters an air conditioned building the sudden drop overthrows his balance.

11 *Complex organisms such as man have built up a complicated system of protective agencies. The allergic response is primarily a protective reaction.* The normal protective mechanisms have been discussed in Section 9. When a noxious substance enters the nose its removal is accomplished by sneezing and the secretion of mucus. The cough, smooth muscle spasm, and increased bronchial secretion of asthma may be looked upon as an attempt to remove a supposed foreign body. Asthma may develop for the first time in connection with a tumor growth in the lung. This is often true asthma and may be relieved by adrenalin or ephedrin. It represents a physiologic reaction, an attempt to remove a foreign body from the lungs. Prompt vomiting which sometimes follows the ingestion of an allergenic food is again a protective response as is the hyperperistalsis



and diarrhea associated with mucous colitis which often follows the ingestion of an allergenic food which the stomach has not repelled. The serous exudation of a contact dermatitis represents an effort to wash away the noxious substance. Lichenification in chronic dermatitis indicates an effort to establish a protective thickening of the skin at the point of contact. Urticaria and angioneurotic edema which involve internal structures probably to nearly as great an extent as they do the visible integument manifest an effort to dilute the allergenic substance in the tissues, thus protecting the living cells.

12. *All allergic manifestations are correlated. They are purposeful reactions. They are pathologic exaggerations or perversions of a normal physiologic function, that of protecting the body against deleterious environmental factors.*

There is abundant indirect evidence indicating a functional connection, either through the nervous system or endocrine system or both, between the various protective tissues or, better, immunity organs, such as the skin and the mucous membranes. Phillips<sup>27</sup> for example believes that pollen hyposensitization can be accomplished more effectively with intracutaneous therapy than with subcutaneous. The fact that the skin reacts positively to a substance such as pollen which causes symptoms on the mucous membrane but not on the skin is further evidence. It seems reasonable to assume some controlling mechanism which integrates the immunity response or protective response of the skin, mucous membranes, glands, smooth muscle, leucocytes and antibodies.

An example might be found in the person who has attacks of diarrhea following therapeutic pollen injections. But here the intestinal reaction does not accomplish the desired purpose. The reactive mechanism is at work but not with effective accomplishment. This brings us to the next point.

13. *The clinical allergic response sometimes manifests itself as a purposeful reaction, purposelessly executed.* The person sensitive to house dust who has asthma from inhaling this allergen is experiencing a purposeful allergic response. It is true that the dust cannot be completely removed in this way and the symptoms therefore continue but the purpose of the symptoms is obvious. The baker with asthma from sensitization to inhaled wheat is another example. But when a wheat sensitive person develops asthma following the ingestion and enteral absorption of bread, the respiratory reaction can scarcely be termed efficacious. In this instance the protective mechanism is in action but is applied through the wrong agency. The reaction is not coordinated.

14. *When the noxious agent acts from within, the same protective mechanism is set in motion. However, in the absence of an external localizing stimulus, the reaction may manifest itself in any or all of the protective tissues or fluids.* The economy of the human body is so arranged that certain tissues serve certain functions. It is supererogatory to detail the various functions, but I would emphasize that when that of protection has been assigned to certain portions of the body the other body cells lose this ability in great measure. Their functions are intrinsic and in a measure much more elementary. For some reason as yet not clearly understood, if the internal tissues or cells come into contact with a foreign protein a certain reaction occurs which, after a preliminary incubation period, makes that protein highly poisonous. The prevailing theories of anaphy-

laxis have attempted to explain why a foreign protein parenterally introduced as through a hypodermic needle becomes poisonous. The present discussion offers nothing new in this regard. Since the Ehrlich hypothesis of antibody formation is the one most generally accepted at the present time and possibly the most easily comprehended we can accept it for this discussion. Suffice it to say that a foreign protein on second entry into the body becomes highly poisonous. This has also been shown to be true for some nonprotein substances.

As long as there is a clearly defined external point of contact the reaction usually takes place in that neighborhood. This is exemplified in the dust-asthma mentioned above. To mention a rather crude example, if in a large city with several fire departments a fire breaks out in a certain locality telephone communication speedily sends the equipment from one of the engine houses to combat the conflagration. But assume a large fire in the center of the city. An automatic system sends a general alarm to all departments but the automatic system is not working perfectly and no definite information is given as to the location of the fire. The engines will dash hither and yon to the several more probable places. This is the situation when the reaction occurs within the body rather than at localizing areas in contact with the environment. When the reaction takes place within the body it may therefore manifest itself by asthma, urticaria, colitis, migraine (localized urticaria or angioneurotic edema), generalized edema (acute anaphylactic shock), or by a combination of two or more of these symptoms.

The reaction is protective but the mechanism does not know which area to protect. Certain areas appear to be more reactive than others.

The tropic importance of a contact factor is well exemplified in the work of Auer,<sup>28</sup> of Vahj Menkin<sup>29</sup> and of Seegal, who have shown in anaphylactic shock that if a given area is first irritated chemically or by local infection antigens accumulate in greater concentration in this area and the local reaction is decidedly more pronounced.

This possibly explains some of the allergic reactions that cannot be attributed to the action of the protective mechanism. It is a matter of common experience that patients with bile tract infections often find that certain specific foods precipitate typical gallbladder attacks. In this case the allergy is an exciting factor, probably attributable to concentration of antigens or to toxins at the site of local infection. The same may be true in cases of peptic ulcer.

One might argue that an inhaled substance should always produce respiratory allergy or a substance ingested should always produce gastrointestinal allergy. It has been demonstrated by the work of Silzberger and myself<sup>3</sup> that foreign allergens may enter the circulation through the lungs or respiratory mucosa, causing no reaction thereon but producing remote lesions in the skin. Certain of the protective tissues may be more reactive than others. An allergenic food may produce gastrointestinal symptoms or after absorption it may be responsible for asthma, migraine or so-called neurodermatitis. The same explanation holds.

I realize that Walzer and his associates have demonstrated that foods when eaten, are absorbed into the blood in a condition still sufficiently like their native state so that they can be demonstrated as having reached the skin by the



play no part here. The reactive mechanism, possibly activated through the autonomic nervous system or the endocrine system or both, receives its stimulus from the higher nerve centers, but the site and basis of the reaction are explained in the same manner as in frank allergy.

18. *The endocrine system probably plays some part in the protective mechanism.* The action of suprarenal cortex in preventing anaphylactic shock<sup>31</sup> and of epinephrine in lessening its severity when it has occurred should be mentioned. It should also be recalled that Cannon<sup>32</sup> has elaborated a theory concerning the function of the adrenal secretion in which he gives it a most prominent place in the protection of the individual against deleterious environmental influences. Epinephrine is a "fight or run" hormone. It speeds the heart, raises blood pressure, relaxes bronchial musculature thereby facilitating respiration, diminishes the blood supply to the skin while increasing that to the internal organs thereby giving additional nutrition to the musculature, discharges glycogen into the circulation, providing an increased blood sugar content for utilization if necessary. Whether the environmental influence calls for combat or for flight the physiologic response to increased epinephrine secretion has provided optimal conditions.

The objection might be raised that epinephrine lessens all of the so-called protective activities which we have described. But it should be borne in mind that the allergic response is a disorganized or disoriented one. We might speak of it as out of proportion to the intensity of the stimulus. It becomes obvious that the adrenal secretion controls the intensity, character and direction of the response. Physiologists have studied the glycogenolytic activity of epinephrine, its action in relaxing smooth muscle spasm, in raising blood pressure and increasing the pulse rate and the like, but there is much that we do not know regarding the effect of this secretion on the tissue cells in general. Epinephrine serves as a balance wheel or a governing factor, controlling the intensity and direction of the allergic reaction. It coordinates it and tends to keep it within normal limits.

19. *The allergic reaction represents a response to an environmental maladjustment.* Richet, visualizing a purely experimental phenomenon, and attempting to explain it in terms of immunology, coined the term *anaphylaxis*, indicating the absence of protection as contrasted with prophylaxis or *favoring protection*, as observed in the development of immunity. Victor C. Vaughan and Wheeler<sup>33</sup> (1907) were the first to state their belief that the fundamental processes at work in immunity and in anaphylaxis were identical. This concept has been generally accepted.

It is at once obvious that in the theory herein presented, the reactive bodies, which are in essence the protective mechanism of the human body, are the same in allergy as in immunity. In the processes of immunity they react with maximum efficiency, thereby protecting the body as a whole. In clinical allergy they do not fail to react as would be indicated by the term anaphylaxis, but they react abnormally. The reaction is, as I have said, disoriented, disorganized. A more appropriate term would be *dysphylaxis* (difficult protection) or *dysphyl-ergy* (labored reaction of protection). The reaction is of normal type, but apparently uncontrolled.

20. *So-called nonspecific therapy or shock therapy, when efficacious, exerts its beneficial effect by stimulating the reactive mechanism to more effective response.* It is a matter of common experience that an allergic individual often loses his allergic manifestation following an acute illness such as pneumonia, typhoid fever, acute appendicitis, any surgical operation or, indeed, simple anesthesia. The same improvement sometimes follows foreign protein therapy such as intravenous typhoid or colon bacillus vaccine, peptone injections, or even the subcutaneous injection of so-called specific or nonspecific vaccine.

It is probable that all of these stimulate the immunity mechanism, the same mechanism which is responsible for the allergic response, in such a way as to make it more effectively responsive, more efficient, at least for a time. Eventually, however, it again loses its acquired effectiveness and allergic symptoms return.

### III. THERE ARE DEGREES OR TYPES OF RESPONSE TO HARMFUL ENVIRONMENTAL CHANGES

21. *Normergy: the normal response.* It would be hazardous indeed to attempt to present a new classification of the phenomena of idiosyncrasy, since there are already so many which use the same terms but interpret them entirely differently. However, there are four terms already in use, although used differently by different writers, which so clearly fit into the hypothesis herein presented that I venture to express my own interpretation of them in illustrating my concept of the manner in which a living body reacts to environmental influences.

The normergic is obviously the person who can always adjust himself satisfactorily to the usual range of deleterious environmental influences. Extremes of temperature and humidity, dietary and inhalant factors, even infection, are well tolerated. Exposure to infection rapidly produces an adequate immunologic response.

Some observers have stated their opinion that allergic individuals are especially resistant to infection. While this statement has not received universal acceptance, it is interesting to speculate on a hyperactivity of the immunity mechanism which would account for this. While the allergic reaction is incoordinated in its clinically recognizable responses, it is nevertheless a rather violent or intense reaction, and it is possible that it is as intense in its serologic and tissue immune response as in its nasal, bronchial, or dermal response. This might result in some increased resistance to infection. On the contrary we should bear in mind Rackemann's observation previously mentioned, that he was unable to find any difference between allergic and nonallergic individuals as regards antibody production.

22. *Allergy; abnormal response to deleterious environmental influences.* The simplest manifestation of an allergic reaction to environment is observed in physical allergy. The person who responds to extreme heat or cold, sudden temperature changes, to ultraviolet radiation or to effort with typical allergic symptoms, such as asthma, vasomotor rhinitis, urticaria, is an allergic individual. His reactive mechanism is broken down and he responds with a misdirected or perverted protective reaction.

The work of Petersen<sup>31</sup> who has shown that deaths from asthma in Chicago are much more likely to occur during a pollen infall, occurring during changes in cyclonic fronts, irrespective of the immediate allergic etiology of the asthma indicates the importance of environmental factors. Apparently some allergic individuals as well as nonallergic tolerate sudden meteorologic changes better than others.

23 *Hyperergy, exaggerated normal reaction to environmental factors* This may or may not be an intermediate stage between normergy and allergy. I have gained the impression in my studies, that allergic persons are more likely to be hyperergic to drugs and the like than are normergies. However, I have made no statistical analysis in this regard.

The person who reacts to extreme degrees of heat, not with asthma or urticaria, but with heat prostration, is hyperergic. He reacts in a normal way, but more violently than the normergic. I would interpret most of Duke's cases of effort syndrome as hyperergic rather than allergic. The person who has adjusted himself to his daily routine may, when inducted into army service and exposed to intensive physical exertion to which he is not accustomed, react with those symptoms which are today well known. He experiences palpitation, tachycardia, tremor, attacks of syncope and the like which are grouped under the designation "effort syndrome," or "soldier's heart." These are the reactions which one normally experiences to unusual or severe effort but which some people, hyperergic, experience following a degree of effort which causes no symptoms in normergies.

The person who develops urticaria from belladonna is allergic. The one who, following normal dosage with belladonna, experiences dryness of the mouth, dilatation of the pupils, or, in extreme cases, atropine convulsions, is hyperergic. Those who sunburn easily are hyperergic. Those who develop asthma following exposure to ultraviolet rays are allergic.

24 *Anergy, absence of reaction* Tissue cells appear to be unable to react at all to certain noxious and deleterious substances with which they come in contact. This includes simple poisons such as bicloride, phenol, acids, alkalis and the like. There is no protective mechanism.

There is no sharp differentiation between these four groups. Workers in tar develop what is known as tar dermatitis. Some do it more easily or sooner than others. These might be classed as hyperergic. But, given sufficiently long contact, nearly all such workers develop dermatitis. This of course will include the normergies, and there is no recognizable division between the two groups. Given sufficient exposure probably 100 per cent will eventually develop symptoms. The reaction is primarily a protective one but manifests itself in an abnormal way and might therefore be called allergic. Here again there is no clear cut point of cleavage between the groups.

If we speak of allergy as a pathologic exaggeration of a normal physiologic response, we might well speak of hyperergy as a normal exaggeration of a normal physiologic response.

In the same way anergy cannot be clearly differentiated. Bicloride of mercury applied to the skin in an allergic individual may produce a contact

dermatitis. It is when this type of chemical comes in contact with the internal tissues, with no external points of contact, as after parenteral administration or sometimes after enteral administration, that anergy manifests itself. The tissue cells themselves appear to have no protective ability although with some of these substances tolerance can eventually be acquired through repeated small dosage.

#### IV. DISCUSSION

The theory herein presented explains the allergic response as an integrated reaction complex, fundamentally protective in nature, but defective in execution since it lacks coordination and a directing influence. It is, in essence, a manifestation of environmental maladjustment. This is in harmony with the idea recently expressed by Gay,<sup>2</sup> "It is our thesis that the ultimate explanation and prevention of a disease depends on ascertaining its external cause. \* \* \* Not only diagnosis but cure and prevention depend on knowledge of the originating impulse. This impulse, animate or inanimate, is the essential element to discover, and when discovered has invariably been found to be external. \* \* \* We have already expressed our prejudice in favor of the eventual explanation of all disease on a basis of external causation. \* \* \* The cause of disease remains predominantly external."

Given sufficiently heavy exposure (contact, meteorologic, food, infectious), 100 per cent of the population is capable of responding abnormally, either with hyperergy or allergy. There appear to be all grades from those who are extremely susceptible to those who are very insusceptible.

Two outstanding factors appear to play a part in determining susceptibility. The first is heredity. This represents either a congenital inability to adjust or a congenital predisposition to maladjust. Such a person is often spoken of as vagotonic. The function of the vagus autonomic system and its relation to allergic manifestations has been discussed.

The second factor is that of the nature of the exciting factor, whether it be protein or not, and the intensity and chronicity of exposure thereto. The mildly allergic individual may become sensitive to a relatively new or strange allergen with which he comes into only occasional contact. The person with high susceptibility may become sensitive to these and also to those substances with which he comes in frequent or chronic contact.

The theory herein proposed very materially simplifies our understanding of allergic therapy. The simplest way to circumvent deleterious reaction to a harmful environment is by avoiding this environment. This is obviously the principle of avoidance in allergic therapy. But where the noxious influence cannot be avoided, acclimatization may be attempted, by exposure of the tissues to constant or repeated contacts. This is the basis of hyposensitization or desensitization and accomplishes results in the same manner that acclimatization to deleterious temperature changes produces increased tolerance.

The process of hyposensitization is in essence a process of acclimation.

This principle places perennial pollen therapy on a rational basis.

The question arises as to why minor allergic individuals become sensitized only to occasional contactants while major allergies display their outstanding idiosyncrasies to frequent or chronic contactants. We have already mentioned the work of Wells who found that guinea pigs born allergic to oats appeared to desensitize themselves when eating oats but showed clear cut allergy thereto when placed upon an oat free diet. It may be that there is as great a tendency to become allergic to allergens to which one is frequently exposed as to those to which exposure is only occasional. The former, however, may very well desensitize by virtue of this frequent exposure, as in the case of the guinea pigs. The minor allergic individual may become hypersensitive to wheat, egg, and milk as easily and frequently as the major allergic, but he fails to manifest symptoms therefrom because he readily desensitizes himself by chronic exposure or acclimatization. The person with an unusually pronounced tendency, the major allergic individual, cannot acclimate himself even with frequent or constant exposure and as a consequence develops symptoms. I would again emphasize that in my experience the major allergic person is also sensitive to occasional contactants, and in roughly the same frequency as the minor allergic. He is fundamentally the same as the minor allergic person in this respect and in addition is unable to acclimate himself to sensitization to frequent contact substances. The minor allergic individual often gives positive skin reactions to substances such as wheat, egg and milk even though these do not cause symptoms. He is allergic but he is acclimatized. This explains many or all of the so called false positive reactions observed in allergic testing.

If susceptibility to the development of allergy varies from zero per cent to 100 per cent one would expect occasionally to find persons who are allergic to practically everything. Such types although fortunately uncommon undoubtedly do exist. I have had occasion to treat a man who was in the produce business, and therefore came into contact with many unusual foods as well as all of the common ones. He was found allergic to 42 different foods, and when tested by the Prausnitz-Kustner technique with the biologic food groups gave positive reactions to 16 of the 30 test extracts. Rinkel<sup>25</sup> has observed certain intractable asthmatic persons who respond with a positive leucopenic index to practically every food with which they are tested. We have been able to confirm this in our own experience. There might be some question as to whether this indicates that they are actually allergic to each of these foods, but there is no gainsaying that, whether allergic or not, they respond to ingestion of all the foods in an allergic manner. Although the reaction may not be specific it is an allergic type of response.

#### CONCLUSION

The theory herein described is presented in an effort to clarify our understanding of the manner and meaning of the allergic response and in the hope that, this being better understood, there may be consequent eventual advances in the therapy of the allergic diseases. If the hypothesis contains an element of truth, it should promote investigation in a field which has received too little attention up to the present. Until now chief effort has been given to neutraliza-



tion or counteraction of a specific response to recognized specific allergens. It seems possible that efforts to develop a nonspecific or physiologic means of controlling the perverted, disoriented reaction of protection so that it may become a normal oriented reaction, might actually lead to the discovery of a remedy which will adequately control the response irrespective of the activating cause.

The two lines of approach which from the theory would appear to offer possibilities, deal with the endocrine system, particularly the adrenals, and the autonomic nervous system. Studies in these two fields have until the present been disappointing, but the possibility always remains that, as was true in the case of Banting and Best's discovery of insulin, a somewhat different method of approach may be more successful. There also remains the possibility of the recognition of a new mechanism not identified with either of these two systems.

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# LABORATORY METHODS

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## A CULTURAL METHOD FOR THE DIAGNOSIS OF GONORRHEA EMPLOYING THE DIRECT OXYDASE REACTION\*

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IN RECENT years the diagnosis of gonorrhea by cultural methods in addition to demonstration of the organism in smears has attracted considerable attention. Fröhlich and Jordan,<sup>1</sup> Hämel,<sup>2</sup> and Schlirf<sup>3</sup> are among those who have investigated the possibilities of cultural diagnosis of gonorrhea recently. McLeod and his associates<sup>4</sup> have no doubt done more in this connection than any other investigators to date. In addition to this they were the first to introduce<sup>5, 6</sup> the use of the direct oxydase reaction for the purpose of detecting gonococcal colonies in mixed cultures.

To our knowledge, only three reports upon the use of the oxydase reaction in the diagnosis of gonorrhea have appeared in the literature since its inception by McLeod. Price<sup>7</sup> and King<sup>8</sup> in England, and Carpenter<sup>9</sup> in this country, have reported the use of this method with favorable results. McLeod<sup>4</sup> reports excellent results on the use of the oxydase reaction in a large series of cases. These favorable reports suggested the possible adaptation of the oxydase reaction to routine laboratory procedure.

In developing a routine laboratory method for the cultural diagnosis of gonorrhea, the first consideration was a suitable medium. It was desirable that this medium should support excellent growth of the organism, and be of such nature or composition that it could easily be duplicated. North gelatin agar (Difco) was found to possess the above qualifications. This medium was enriched by the addition of 10 per cent sheep blood while hot. This gave a chocolate medium upon which the gonococcus could be grown with little difficulty. The composition of this medium is always constant and comparable results should be obtainable anywhere with its use. The medium was sterilized and stored in 100 c.c. flasks in the refrigerator, and when ready to use was liquefied, the blood added, and the plates poured. In this way a fresh, moist surface for growth was afforded the gonococcus.

The source of material for culture was some distance from the laboratory, and a point of difficulty was encountered when the problem was first begun. The swabs of the exudates were transported to the laboratory where the plates were inoculated. This method proved to be unsatisfactory since the exudate dried rapidly on the swabs. It was noted that the less exposure to drying and lowered

\*From the Department of Pathology, Ohio State University.  
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temperatures to which the material was subjected, the better the possibility of growth. When this fact had been ascertained, a method was devised whereby the plates could be taken to the source of the material, inoculated, and returned to the laboratory without undue exposure. A corrugated paper box large enough to hold several dozen Petri plates was lined well with cotton batting. The plates were put in this box, packed well with cotton, and the box sealed. In this way the plates could be transported to the patients, inoculated, and returned to the laboratory for incubation without any undue exposure. Undoubtedly, however, the best procedure is to have the patient come to the laboratory.

The plates are streaked with the swab containing the exudate, after which they are incubated at 37° C. Colonies grow on this medium within twenty-four hours and are considered mature within from forty-eight to seventy-two hours.



Fig. 1—*Gonococcus* in mixed culture on heated blood agar, as usually obtained from the female urethra. Before treatment with tetramethyl *p*-phenylenediamine hydrochloride.

The plates are now covered with a freshly prepared 1 per cent aqueous solution of tetra methyl para phenylene diamine hydrochloride,\* which is immediately poured off. Medium sized convex and translucent colonies which rapidly turn a bright purple color, are gonococci, if upon microscopic examination they are gram negative diplococci.

Carpenter† considers the dimethyl para-phenylene diamine superior to the "tetra methyl" compound, believing the latter to be less toxic but staining the medium so deeply that there is frequently difficulty in distinguishing a gonococcus colony.

In the work discussed in this paper no difficulty was encountered in this connection. True, the medium does turn a deep purple color after a few minutes, but there is sufficient time to detect the gonococcal colonies and "fish" them for

\*Prepared by Eastman Kodak Co. (Organic chemical division.)

†Personal communication.

transplants and microscopic examination before the medium is dark enough to make the colony indistinguishable.

In the great majority of cases where a positive oxydase reaction was secured, the organism was a gram-negative diplococcus when checked by staining and microscopic examination.

The practical importance of the oxydase reaction in the detection of gonococcal colonies lies in the fact that one can readily observe the presence of gonococcal colonies in a heavily mixed or contaminated culture such as is obtained from the urethra of the female, whereas by ordinary methods this would be a long and laborious procedure.



Fig. 2.—The same plate culture (Fig. 1) after treatment with tetramethyl-p-phenylenediamine hydrochloride. The dark colonies are oxydase positive gonococci.

#### SUMMARY OF TECHNIC

*Medium.*—North gelatin agar (Difco) prepared as directed on container. Heated to 75° C. and 10 per cent sheep blood is added, which gives a chocolate medium; allowed to solidify and then inoculated.

*Incubation.*—At 37° C. for forty-eight hours.

*Identification of Gonococcal Colonies.*—One cubic centimeter of the dye compound is poured on the surface of the medium to flood the colonies, and is poured off immediately. The gonococcal colonies turn a bright purple color in a few seconds.

#### RESULTS OBTAINED IN THE CULTURAL DIAGNOSIS OF GONORRHEA IN THE MALE

Males coming to the venereal disease clinic and presenting the clinical symptoms of gonorrhea were examined culturally for diagnosis. The majority of the patients observed were in the acute stage and exhibited purulent discharge. Seventy-seven patients were examined. The results are tabulated in Table I.

TABLE I  
COMPARISON OF SMEARS AND CULTURES OF THE MALE URETHRA

NEGATIVE SMEAR	NEGATIVE CULTURE	NEGATIVE SMEAR	POSITIVE CULTURE	POSITIVE SMEAR	NEGATIVE CULTURE	POSITIVE SMEAR	POSITIVE CULTURE
	22.0%		10.3%		7.7%		60.0%

In Table I it may be noted that in a small number of cases a positive culture was obtained when the smear was negative. In two cases a positive smear was accompanied by a negative culture.

#### RESULTS OBTAINED IN THE CULTURAL DIAGNOSIS OF GONORRHEA IN THE FEMALE

Females undergoing treatment for gonorrhea at the venereal disease clinic were examined culturally for progress of treatment. The majority were chronic cases of gonorrhea. Fifty-nine patients were examined. The results are given in Tables II and III.

TABLE II  
COMPARISON OF SMEARS AND CULTURES OF THE FEMALE URETHRA

NEGATIVE SMEAR	NEGATIVE CULTURE	NEGATIVE SMEAR	POSITIVE CULTURE	POSITIVE SMEAR	NEGATIVE CULTURE	POSITIVE SMEAR	POSITIVE CULTURE
	52.5%		10.2%		0		37.3%

Positive cultures were obtained in all cases showing positive urethral smears and in six cases showing a negative urethral smear. In no cases were there negative cultures with positive smears.

TABLE III  
COMPARISON OF SMEARS AND CULTURES OF THE FEMALE CERVIX

NEGATIVE SMEAR	NEGATIVE CULTURE	NEGATIVE SMEAR	POSITIVE CULTURE	POSITIVE SMEAR	NEGATIVE CULTURE	POSITIVE SMEAR	POSITIVE CULTURE
	49.2%		32.2%		0		18.6%

Positive cultures were obtained in a considerable number of cases in which the cervical smear was negative.

The data presented in Tables II and III indicate that the cultural method is superior to the microscopic method in the diagnosis of gonorrhea in the female. This was most marked in the examination of the cervical exudates. Smear positive 18.6 per cent; culture positive 50.8 per cent.

#### DISCUSSION

Cultural methods accompanying physical examination of the urogenital tract furnish an index of cure and greater accuracy in the diagnosis of gonorrhea. The value of the cultural method is further enhanced by the oxydase reaction, which demonstrates the presence or absence of gonococcal colonies in a mixed culture such as is usually obtained from the female urethra. The microscopic examination of all the colonies in such a culture would be a time-consuming and tedious task, whereas by the use of the oxydase reaction the presence of gonococcal colonies may be quickly determined.

transplants and microscopic examination before the medium is dark enough to make the colony indistinguishable.

In the great majority of cases where a positive oxydase reaction was secured, the organism was a gram-negative diplococcus when checked by staining and microscopic examination.

The practical importance of the oxydase reaction in the detection of gonococcal colonies lies in the fact that one can readily observe the presence of gonococcal colonies in a heavily mixed or contaminated culture such as is obtained from the urethra of the female, whereas by ordinary methods this would be a long and laborious procedure.



Fig. 2.—The same plate culture (Fig. 1) after treatment with tetramethyl-p-phenylenediamine hydrochloride. The dark colonies are oxydase positive gonococci.

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## ANIMAL GROWTH AND SPACE RESTRICTION\*

I. NEWTON KUGELMASS, M.D. AND EMMA LOUISE SAMUEL, M.A.,  
NEW YORK, N. Y.

**N**UTRITIONAL studies in progress for several years have revealed that space restriction is a factor involved in the character of growth and development of young animals. In three sets of experiments on young rats maintained on a balanced and adequate basic dietary it was found difficult to obtain satisfactory growth. After eliminating some factors—good progeny, a complete dietary, freedom from infection, favorable hygiene, adequate sunshine—spatial restriction appeared to be the detrimental factor to growth and development. With this in view the experiment was repeated with another series of animals, maintaining all conditions constant excepting the volume of space occupied per animal.

### METHOD OF PROCEDURE

White albino rats of known pedigree stock, five weeks old were selected for study in small and large cages respectively, each animal having available 120 c. m. space in small cages, 430 c. m. space in the large cage. The cages used were standard make of galvanized wire netting with double bottom. The diet given below was prepared in required amounts for the twenty-four hour period, and placed in cages mornings, water was added ad lib, weights were taken weekly, and those that thrived were put into the activity cage. The lima beans and potatoes were cooked for three hours at forty pounds' pressure and the other ingredients were added later. These mixtures were prepared on the average of three times a week, kept in refrigeration between 40° to 45° F. and fed daily.

TABLE I  
DIETARY COMPOSITION

FOOD	WEIGHT	PRO	CARBO	FAT	CALORIES	BASIC
Khm	20 gm	5.2	7.6	3.0	105.0	3.3
Potato	100 gm	2.5	20.9	0.1	96.9	7.7
Beans (lima)	60 gm	10.8	39.1	0.9	210.0	24.0
Dextrin	3 gm	---	2.7	-	17.0	---
Agar	2 gm	-	-	-	---	---
		19.5	70.8	6.0	428.9	

The groups of animals studied in small and large cages were compared quantitatively from the standpoint of relative activity. The animals confined in small cages were entirely too feeble to be placed in the activity cage and so

\*From the Heelcher Institute  
Received for publication May 4 1935



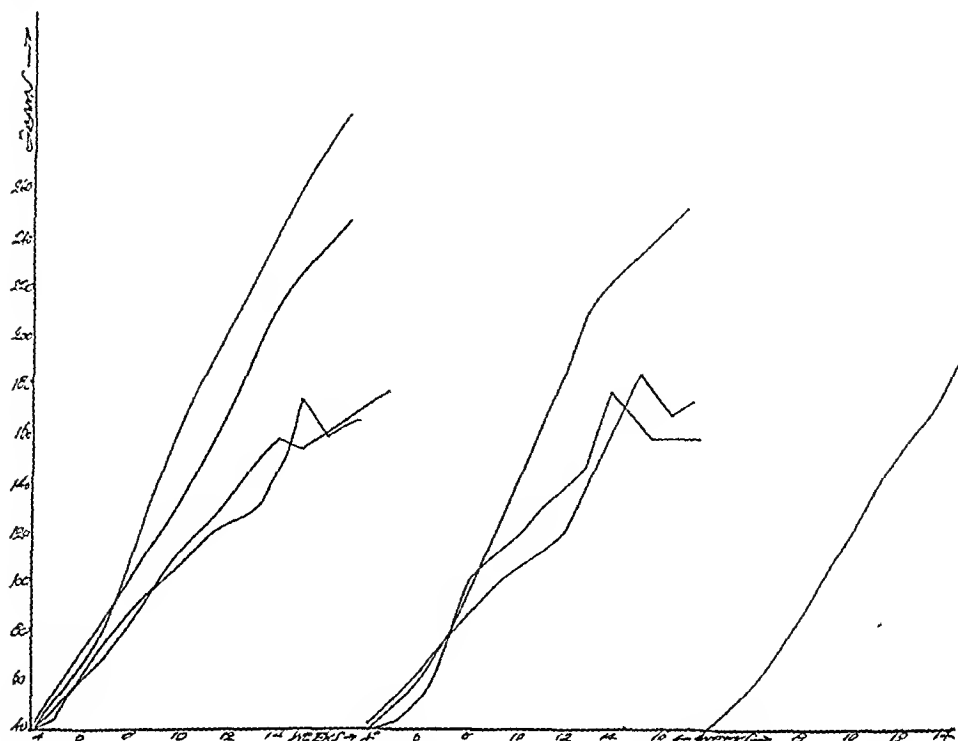


Chart 1.—The growth curves of rats maintained on normal diets without space restriction.

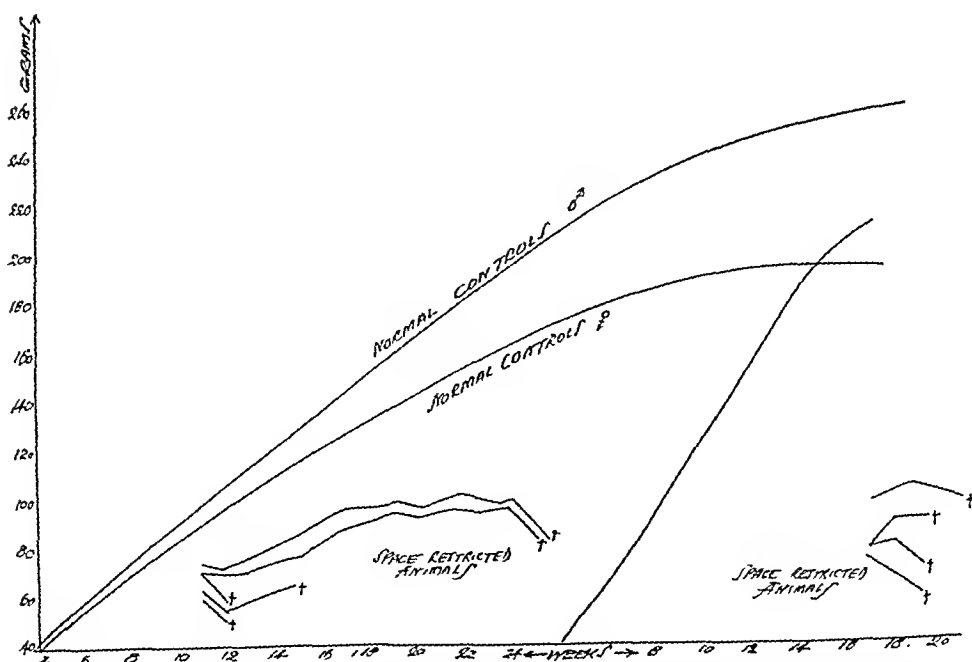


Chart 2.—The growth curves of rats maintained on normal diets with space restriction.  
 †Normal controls.

only results of the animals in the large cages are given below. Each animal was first confined in the activity cage without freedom and twenty four hours later the same animal was maintained in activity with voluntary periods of freedom.

TABLE II  
ACTIVITY VALUES COMPARATIVE RESULTS OF ANIMALS ON COMPULSORY AND INTERMITTENT ACTIVITY

RAT NO	CONTINUOUS ACTIVITY			INTERMITTENT ACTIVITY		
	WT LOSS	FOOD CONSUMED	QUOTIENT	WT LOSS	FOOD CONSUMED	QUOTIENT
048	8 gm	6 gm	2051	3 gm	26 gm	2092
049	5 gm	12 gm	4511	6 gm	20 gm	1651
050	2 gm	15 gm	2158	2 gm	20 gm	750
051	6 gm	16 gm	2656	3 gm	20 gm	3526
052	6 gm	9 gm	2641	2 gm	25 gm	1810
053	1 gm	6 gm	3013	1 gm	25 gm	722
054	6 gm	7 gm	2203	2 gm	20 gm	2212

TABLE III  
COMPARATIVE RESULTS OF ANIMALS LIVING IN SMALL AND LARGE CAGES

	SPACE VOLUME PER ANIMAL	DATE OF CUL	LIFE SPAN	ACTIVITY VALUE PER DAY	NO ANIMALS PER EXPERIMENT	INITIAL WEIGHT
Small cage	120 c m	1 gm	8 wk	Feeble	9	71 gm
Large cage	420 c m	34 gm	18 wk	2500 rev	7	40 gm

# RESULTS

The animals maintained in the small cages occupying on the average a space of 120 c m per rat failed to gain weight, grow, or develop consistently. One of them died in the third week, the second in the fifth week, a third was put on another normal diet, died nevertheless in the seventh week. In another series of animals six weeks old, the initial weight averaging to 67 gm, the life span was fifteen weeks with a change in weight of 10 gm of their initial weight. Another series six weeks old, weighing between 55 and 88 gm, the fourth remaining alone on another normal diet, continued to live. Their average food consumption was 34 gm per rat per day.

The animals maintained in a large cage, occupying a volume of 420 c m per animal, all thrived on the same dietary as those in the small cages. The rats were four weeks old, averaging 40 gm with an average daily food consumption of 92 gm per rat. These two groups of animals in large and small cages respectively on the same dietary showed a striking difference in the character of their growth and development. The animals confined in the small space died within two or three months as compared with those in the large space which continued to live. In a previous communication we have demonstrated that the high forming dietary is conducive to great activity with marked liberation of energy in comparison with other dietary. Space restriction on such buoyancy forming dietary is evidently not favorable for development.

The animals maintained in a spacious environment were further tested from the standpoint of compulsory and leisure activity. The average amount of food consumed was 39 gm per rat per day, the average loss of weight per rat in the

compulsory group was 5.3 gm. in comparison with the leisure group which was 2.8 gm. The revolutions of the compulsory group were 2,828 in comparison with the leisure group which were 1,706 revolutions. The average of food consumption of the compulsory group was 10 gm. per rat per day in comparison with 27 gm. of the leisure group of rats.

#### CONCLUSIONS

1. A comparative study of two groups of young rats maintained on a normal base forming dietary revealed that the volume of space occupied was a factor involved in growth and development.

2. The animals maintained in a limited volume of space failed to thrive in comparison with those reared in a spacious environment.

3. The life span of the animals confined in a restricted space was short in comparison with those reared in abundant space.

4. The average food consumption of the animals confined to continuous activity was considerably less than those offered relative freedom activity.

# DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDLIFF, M.D., ABSTRACT EDITOR

**PNEUMONIA, LOBAR:** Occurrence of Myelocytes in Peripheral Blood in. Stephen, D. J. *Am. J. M. Sc.* 188: 332, 1934

In 43 unselected cases of lobar pneumonia, the white blood cell response was studied in detail, using both fixed and supravital stained preparations

In the majority of patients, small numbers of myelocytes were frequently found in the peripheral blood during the acute febrile period of the disease

In patients who recovered, a consistent, and frequently striking, shower of myelocytes was observed in the peripheral blood during the period immediately following crisis and lysis.

It is suggested that the postfebrile myelocyte shower may be due to the stimulating effect of nuclear material liberated during resolution of the pneumonic exudate.

**PNEUMONIA:** Cytology of Pleural Effusion Studied With a Supravital Technic, Scott, T. F. McNair, and Finland, M. *Am. J. M. Sc.* 188: 322, 1934

A supravital technic was used to study the cytology of 53 pleural fluids from 32 patients with pneumococcus lobar pneumonia. The cell types encountered are described. Their occurrence and frequency are correlated with the outcome of the effusion and the course of the pneumonia.

The cellular content of the infected fluids consisted almost exclusively of polymorpho nuclear neutrophils in various stages of degeneration. In uninfected fluids the predominant cells, in the beginning, were active polymorphonuclear neutrophils, but these decreased in number during the first week at which time monocytes and macrophages appeared in the fluid. Later in the disease, after crisis had taken place, lymphocytes began to appear in these sterile fluids.

Moderate to marked eosinophilia was noted in three cases. This occurred during the third week, or later, after the onset of the pneumonia.

**TUBERCULOSIS,** Clinical Study of Allergy and Immunity, Karan, A. A. and Danford, V. H. *Am. Rev. Tuberc.* 30: 320, 1934.

An attempt to correlate the intensity of the intracutaneous tuberculin reaction with the clinical progress, in a group of 40 tuberculous patients receiving collapse therapy, gave negative results.

The intracutaneous tuberculin test, before collapse therapy was instituted in this group of patients, failed to reveal any correlation between the intensity of the reaction and the type of disease.

Graphs of the successive reactions in each patient did not disclose any typical curve in patients who improved and in those with progressive disease.

**ANEMIA:** Evanescent Effect of Intratibial Injections of B. Welchii Toxin in Rabbits, Goldwater, L. J., Connery, J. E., and Heumann, H. *Am. J. M. Sc.* 188: 319, 1934.

In the authors' search to find a suitable method for assaying the potency of substances effective in the treatment of the macrocytic hyperchromic anemias they attempted to induce an anemia in rabbits by a single intratibial injection of hemolysin free B. Welchii toxin. Since the changes in the blood of the test animals were quite transient and since the blood picture of the test animals rapidly returned to a condition similar to that seen in the control animals, this method was deemed unsatisfactory for their purposes.

**TUBERCULOSIS: Leukocyte Count During Artificial Pneumothorax Treatment and Lung Expansion, Deegan, J. K. Am. Rev. Tuberc. 30: 256, 1934.**

The Medlar interpretation of blood counts (see below) in cases of far-advanced pulmonary tuberculosis subjected to collapse therapy is a valuable guide in the clinical administration and control of these patients.

TYPES	WHITE BLOOD CELLS	POLYMORPHO-NUCLEARS	LYMPHOCYTES	MONONUCLEARS
Septic type, abscess-formation. New tubercle-formation if mononuclears above 9 per cent	Usually above normal. May have leucocytosis or leucopenia	Above 65 per cent	Below 25 per cent	Usually above 8 per cent
Hyperplastic type, new tubercles formed but not undergoing abscess-formation. 30 per cent lymphocytes = healing tendency	Normal limits	Below 60 per cent	25 per cent or more	Above 10 per cent
Nonseptic type	Never over 10,000	Lymph per cent = or exceeds polys per cent		Below 10 per cent

**RETICULOCYTES: Lack of Effect of Liver Treatment on the Circulating Reticulocytes in the Pigeon, Heimann, H., Connery, J. E., and Goldwater, L. J. Am. J. M. Sc. 188: 343, 1934.**

From these experiments the following conclusions seem warranted:

1. The percentage of the circulating reticulocytes in the blood of the pigeon is subject to wide fluctuations.
2. The various test substances used in this study, including the "incomplete" diet, had no significant effect upon the percentage of the circulating reticulocytes in the pigeon.
3. In the authors' hands this technic does not lend itself to the biologic assay of the potency of materials used in the macrocytic hyperchromic anemias.

**BICHLORIDE POISONING, TREATMENT OF: Study of 46 Cases, Porter, W. B., and Simons, C. E. Am. J. M. Sc. 188: 375, 1934.**

*Treatment in Detail:* 1. Immediate gastric lavage with a saturated solution of sodium bicarbonate, temperature 100° F. This is continued until the return fluid is clear. The lavage is repeated every twelve hours for the first five days and is continued over a longer period if the chemical analysis of the washings shows mercury.

2. Morphine sulphate is administered immediately after the primary gastric lavage. This is repeated at intervals dependent upon the degree of shock, vomiting and pain; but the principle should be to relieve discomfort, retching and shock.

3. Sodium bicarbonate, 500 c.c. of a 5 per cent solution, is given intravenously immediately after the lavage, and 1,000 c.c. of normal saline solution are administered subcutaneously. As long as vomiting persists the same amount of each solution is repeated every twelve hours.

4. Sodium bicarbonate 5 gm. is given orally every three hours during the day and every four hours during the night. The amount of bicarbonate may be varied provided the urine is kept alkaline to litmus.

5. The total daily fluid intake must be at least 5,000 c.c. for an adult, and this amount must be maintained by oral, subcutaneous, or intravenous route, dependent upon the ability of the patient to retain the substances taken orally. Preference is always given the oral method.

6 The daily diet is orange juice 300 cc milk 1,000 cc and beta lacta 100 gm. One egg daily is added as soon as vomiting is controlled. The feedings should be given every three hours. If vomiting interrupts the feeding schedule 10 per cent glucose solution is given intravenously in amounts sufficient to maintain an intake of at least 1,000 calories. The bicarbonate solution can be made with the glucose solution and the injection given by the intravenous "drip" method. After the first week adequate nutrition and medication are in some cases interrupted by the development of a severe stomatitis and esophagitis. In two of the patients of this series a gastrostomy was done and we feel that it was the deciding factor in recovery. All the food and medicine were given through the gastrostomy tube and only water was given orally.

7 A colonic irrigation, using 5 per cent sodium bicarbonate solution is given daily and is continued until recovery is assured.

While there is no known specific antitoxin for corrosive sublimate poisoning, yet a therapeutic scheme which emphasizes the use of sodium bicarbonate solution for gastric lavage, for colonic irrigation, and for dosage in amounts sufficient to maintain urine alkaline to litmus apparently reduced the mortality and morbidity in this series of patients so treated.

A concept of the mechanism of renal insufficiency in mercury nephrosis is presented and a tentative explanation of the mode of action of alkalinization in preventing uremia is offered.

#### ENCEPHALITIS Acute, Pathologic Changes of the St. Louis Type, McCordock H. A. Collier, W., and Gray, S. H. J. A. M. A. 103: 822, 1934

The severe cases of the St. Louis type differed from the lethargic type in the following respects:

1 The meninges showed more intense infiltration with mononuclear cells than usually was found in the lethargic type.

2 Degenerative changes in the nerve cells were more frequent and neuronophagia was more marked.

3 The inflammatory foci were more widespread throughout the brain, often occurring in great numbers in the cerebral cortex and were not restricted to the midbrain or basal nuclei.

4 The cranial nerve nuclei, especially the third, rarely showed degenerative changes such as are frequent in the von Economo type.

5 There was more extensive involvement of the spinal cord in the St. Louis type.

As the epidemic progressed the lesions became less intense and not so widely scattered. Many of the milder cases showed about the same degree and intensity and a similar distribution in the midbrain, basal nuclei and pons as was seen in the lethargic type. Therefore, as far as these milder cases are concerned it is impossible to differentiate them with any degree of certainty from the lethargic type on the basis of the pathologic lesions alone.

The lesions described by the Japanese in their type B encephalitis are similar in many respects to those of the St. Louis type. The intensity of the meningeal infiltration, the frequency and distribution of the focal collections of cells, the absence of cranial nerve nuclei involvement, and the frequent presence of lesions in the spinal cord, are strikingly similar in both of these types. The onset of softening (associated with the disease) and the retrogressive changes in the cellular foci (glial nodules) which they described were infrequent in our material.

The essential pathologic process in the St. Louis type of encephalitis is a nonsuppurative inflammation of the nervous system characterized by intense vascular congestion, cellular infiltration, and degenerative changes in the nerve cells.

Severe examples of the disease which closely resemble the Japanese type B can readily be distinguished from the lethargic type although the milder cases cannot be differentiated from the latter on the basis of the pathologic lesions alone.

**TYPHOID:** H and O Agglutination of *B. typhosus* in a Group of Suspected Typhoid Cases and in a Group of Unsuspected Individuals, Lewin, W. S. A. M. J. 8: 731, 1934.

In a series of 32 proved typhoid cases "H" agglutination in a serum dilution of 1 in 100 was positive in 81 per cent. In 62 nontyphoid cases only one showed "H" agglutination in a dilution of 1 in 100; the serum from this same case agglutinated *B. typhosus* 0 901 in dilutions up to 1 in 400, and there was a history of three weeks' illness, possibly typhoid, previous to admission to the hospital. Referring to the diagnostic titer for "H" agglutination in typhoid, Felix states: "In 'H' agglutination even a weak reaction in a serum dilution of 1 in 100, corresponding to + or  $\pm$ , may be considered 'positive.' " The author's findings indicate that 1 in 100 is a satisfactory diagnostic titer for "H" agglutination in typhoid fever, provided the clinical signs and symptoms and the past history of the patient are considered.

The higher percentage of "O" agglutination results in typhoid cases with negative blood cultures as compared with those cases with positive blood cultures tends to confirm the opinion formed by some observers that "O" agglutination in high titer is a good prognostic sign.

The results of the tests indicate that, while 1 in 500 as a diagnostic "O" titer, as suggested by Dulaney, is too high, that of 1 in 100, as recommended by Felix, is too low. Lewin considers that a strong (+++) "O" agglutination in a serum dilution of 1 in 200 is a more acceptable minimum diagnostic titer.

The results obtained with the strains *B. typhosus* "H" and "O" have proved of greater diagnostic value than the polyvalent saline suspension. Emerging from this investigation, agglutination of the "H" strain to a titer of 1 in 100, and the "O" to a titer of not less than 1 in 200, is suggested as acceptable diagnostic titers. In the case of initial negative results, the test should be repeated with a specimen of serum collected at a later date.

"H" and "O" agglutination results in suspected typhoid cases must be considered together with the past history of the patient.

**TUBERCLE BACILLI, New Medium for Rapid Cultivation of, Guernon, A. Am. Rev. Tuberc. 30: 510, 1934.**

The medium suggested is prepared as follows:

Agar 0.86 per cent; same amount of peptone; 1.73 per cent potato meal; 3.48 per cent glycerin and 26.09 egg, with the addition of gentian violet 1-30,000 put up in tubes, as a more convenient method.

1. Dissolve, as completely as possible, by stirring with a glass rod, 10 gm. of potato meal and 5 gm. of peptone in a beaker containing 385 c.c. of distilled water.

2. Add 5 gm. of agar, and heat mixture to boiling point, till agar is completely dissolved.

3. Cool to 65° C., then add a mixture containing 20 c.c. of glycerin, 150 c.c. egg and 1.8 c.c. of a 1 per cent alcoholic solution of gentian violet (previously filtered).

4. Filter mixture.

5. Tube in quantities of 7 or 8 c.c. and slant.

6. Inspissate for two hours at 70° C. on the first day and one hour on second day.

As contamination occurred only during the inoculation of the tubes, the medium was not tested for sterility.

Technic: Fresh sputum, containing varying numbers of bacilli, was treated according to Petroff's (1915) method: 4 per cent sodium hydroxide incubated, centrifugated and an addition of 7 drops of normal hydrochloric acid to every cubic centimeter of the mixture, again centrifugated for fifteen minutes and the sediment planted, a single loop on the surface of the medium. Ordinary corks were used, sealed with paraffin and incubated, sloping surface down. The last step is important in order to prevent excessive moisture.

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